Poster number: 1522

Suzanne van Asten, MD, Department of Plastic Surgery
5323 Harry Hines Blvd #F4.306, Dallas, Texas 75390-9132 Office:
214-648-9114 Fax: 214-648-2550
Suzanne.vanasten@utsouthwestern.edu

The Infected Diabetic Foot: 16S rRNA to Characterize the Ecology of Diabetic Foot Osteomyelitis

Suzanne AV Van Asten[1,2], Javier La Fontaine[1], Edgar JG Peters[2], Kavita Bhavan[3], Paul J Kim[4], Lawrence A Lavery[1]

[1] Department of Plastic Surgery, University of Texas Southwestern Medical Center, Dallas, TX, USA
[2]Department of Internal Medicine, VU University Medical Center, Amsterdam, the Netherlands
[3]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA
[4]Department of Plastic Surgery, Georgetown University Medical Center, Washington, DC, USA

Introduction

Diabetic foot osteomyelitis (DFO) develops in approximately 44 to 68% of patients with diabetes mellitus admitted to the hospital with a diabetic foot infection (DFI) [1] and is the leading cause of amputation among such patients [2]. The microbiologic spectrum of DFO seems to be similar to deep diabetic foot soft tissue infections [3] and primarily consists of gram-positive bacteria. However, conventional culture techniques focus on organisms easily cultured using traditional microbiological evaluations and are limited by the time required for organisms to grow [4]. Little is known about the diversity of bacteria in DFO and the contribution of anaerobic and fastidious organisms to these infections [5]. This study aimed to better characterize the bacterial ecology of DFO using a modern 16S rRNA gene sequencing approach.

Results

- Primary genus with both techniques *Staphylococcus* spp.
- Most prevalent population after that *Corynebacterium* spp.
- Significantly more anaerobic pathogens with sequencing approach (86.9% vs 23.1%; p=0.001)
- Significantly more gram positive bacilli with sequencing approach (78.3% vs 3.8%; p<0.001)
- More polymicrobial infections with sequencing approach (91.3% vs. 64.0%; p=0.125)
- *Staphylococcus* spp. were detected in all of the negative samples, with an average contribution of 21.8% to the total bacterial population.

Methods

- We obtained 34 bone samples from patients admitted with a moderate/severe diabetic foot infection according to IDSA guidelines [6].
- Exclusion criteria: other infectious diseases, previously diagnosed DFO in study foot, immunosuppressive therapy, malignancies, renal disease stage 4/5.
- We performed a percutaneous biopsy using a 16 gauge Jamshiti needle introduced at least 2 cm from the ulcer site (n=7) [7] or we obtained intraoperative bone samples from the patients that required surgical debridement or amputation (n=27).
- Part of the bone sample was sent to to the laboratory for conventional culturing and histopathological tests and a part was promptly stored at -80°C until the end of the study.

16S rRNA Sequencing

- We extracted DNA using the Roche High Pure PCR Template kit (Roche Life Sciences, Indianapolis, Indiana).
- We amplified sequences with the HotStar Taq Plus Master Kit (Qiagen Inc, Valencia, CA).
- We denoised the DNA and removed chimeric sequences (8).
- We used the Illumina MiSeq (Illumina, Inc. San Diego, CA) personal Sequencer in collaboration with Pathogenius labs (Pathogenius, Lubbock, TX) to assess the distribution of 16S rRNA gene sequences.
- We clustered the sequences into operational taxonomic units using the UPARSE algorithm (9).
- We ran the centroid sequence from each cluster against the NCBI database, March 2015.

Discussion

- *Staphylococcus* spp was detected in 89.6% (26 of 29) of the sequenced samples, its average contribution to the total bacterial population was the highest of all the genera.
- *Corynebacterium* spp. is a prevalent population in DFO as previously reported [10] , however its pathogenic role remains unclear.
- Anaerobes may play a bigger role in DFO, studies of osteomyelitis in other areas have reported the same finding [11].
- Discrepancies between techniques might be caused by the great plate count anomaly.
- Bacterial diversity may contribute to the variation in medical success rates of DFO [12].

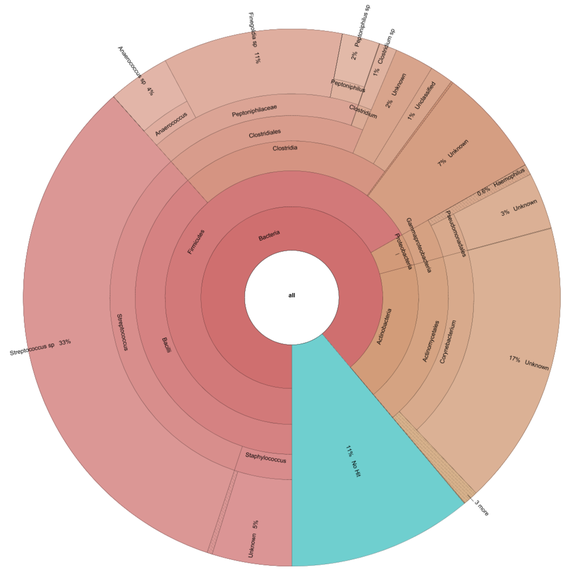

Figure 1. Pie chart of hierarchical 16S RNA data of one of the bone samples

Table 1. Bacterial genera identified in 23 positive bone samples

Genera*	Samples	Avg %	SD	Min-max %
No Hit	22	15.6	26.1	0.03-87.1
Staphylococcus spp.	20	28.6	34.6	0.17-98.8
Corynebacterium spp.	18	7.0	10.7	0.01-33.8
Peptoniphilus spp.	17	2.3	3.1	0.01-11.7
Unknown Firmicutes	16	13.1	19.5	0.02-55.6
Finegoldia spp.	15	8.1	11.8	0.17-44.6
Unknown Clostridialis	14	3.3	8.4	0.02-32.1
Streptococcus spp.	13	20.1	19.5	0.03-57.9
Anaerococcus spp.	12	8.2	8.7	0.06-27.6
Propionibacterium spp.	11	0.9	1.7	0.002-5.0
Clostridium spp.	9	0.9	1.1	0.008-3.3
Unknown Dermabacteriae	8	0.1	0.1	0.03-0.3
Unclassified Clostridialis	8	1.1	1.6	0.008-3.9
Unknown Clostridia	8	2.0	2.1	0.03-6.8
Porphyromonas spp.	7	1.8	1.7	0.03-4.8
Unclassified Clostridia	7	1.3	1.5	0.004-3.6
Unknown Bacteria	7	2.2	5.1	0.01-13.8
Actinomyces spp.	6	1.0	1.8	0.003-4.7
Enterobacter spp.	6	6.0	11.3	0.10-28.8
Prevotella spp.	5	3.2	5.5	0.04-13.0
Helcococcus spp.	5	1.2	1.5	0.05-3.8
Pseudomonas spp.	5	20.8	42.8	3.90-52.6

**Genera sequenced that occurred in at least 21.7% (5 of 23) of the positive bone samples. The genera are sorted by the number of bone samples in which they were detected. Avg %, average percentage each genus contributed to its positive samples. SD, standard deviation of the percentages. Min-max %, range of the percentages. No hit, sequence has no match with the sequences in the NCBI database.*

Table 2. Bacterial genera in diabetic foot osteomyelitis with two culturing techniques

Conventional culture techniques		16S rRNA sequencing*	
Pathogens	Overall (%) Total number of patients = 26	Pathogens	Overall (%) Total number of patients = 23
Gram-positive cocci	20 (76.9)	Gram-positive cocci	23 (100.0)
S. aureus, total	13 (50.0)	Staphylococcus spp.	20 (86.9)
S. aureus resistant to methicillin	3 (11.5)	S. aureus resistant to methicillin	Not tested
Coagulase-negative staphylococci	11 (42.3)	Coagulase-negative staphylococci	Not tested
Streptococcus spp.	6 (23.1)	Streptococcus spp.	13 (56.5)
Enterococcus spp.	2 (7.7)	Enterococcus spp.	0
		Unknown Dermabacteriae	8 (34.8)
Gram positive bacilli	1 (3.8)	Gram positive bacilli	18 (78.3)
Corynebacterium spp.	1 (3.8)	Corynebacterium spp.	18 (78.3)
Gram-negative bacilli	13 (50.0)	Gram-negative bacilli	10 (43.5)
P. aeruginosa	4 (15.4)	Pseudomonas spp.	5 (21.7)
S. maltophilia	1 (3.8)	S. maltophilia	0
Proteus spp.	1 (3.8)	Proteus spp.	0
		Enterobacter spp.	6 (26.1)
Anaerobes	6 (23.1)	Anaerobes	20 (86.9)
Facultative anaerobes	3 (11.5)	Facultative anaerobes	17 (73.9)
		Propionibacterium spp.	11 (47.8)
		Actinomyces spp.	6 (26.1)
		Helcococcus spp.	5 (21.7)
Obligate anaerobes	3 (11.5)	Obligate anaerobes	20 (86.9)
		Peptoniphilus spp.	17 (73.9)
		Finegoldia spp.	15 (65.2)
		Anaerococcus spp.	12 (52.2)
		Porphyromonas spp.	7 (30.4)
		Prevotella spp.	5 (21.7)
		Unknown Firmicutes	16 (69.6)
		Unknown/Unclassified Clostridia	15 (65.2)
		Unknown/Unclassified Clostridialis	22 (95.7)
		Clostridium spp.	9 (39.1)
Polymicrobial infections	16 (64.0)	Polymicrobial infections	21 (91.3)
Unknown Bacteria	NA	Unknown Bacteria	7 (30.4)

** Genera sequenced that occurred in at least 21.7% (5 of 23) of the positive bone samples. Data are number of patients (%).*

Table 3. Bacterial genera identified in 6 negative bone samples

Genera*	Samples	Avg %	SD	Min-max %
Staphylococcus spp.	6	21.8	39.3	0.05-100.0
No Hit	4	49.9	40.8	5.07-97.9
Corynebacterium spp.	3	3.8	1.6	1.99-5.0
Propionibacterium spp.	3	5.9	9.5	0.35-16.8
Streptococcus spp.	3	12.2	15.3	1.37-29.7
Anaerococcus spp	3	4.4	5.6	1.12-10.9
Finegoldia spp.	3	6.8	3.2	3.14-8.9
Peptoniphilus spp.	3	1.9	1.4	1.02-3.5
Unknown Firmicutes	3	7.2	9.1	0.03-17.4
Enterobacter spp.	3	0.2	0.2	0.02-0.5
Unknown Microbacteriaceae	2	7.6	10.4	0.27-15.0
Unknown Enterobacter	2	0.4	0.5	0.02-0.7
Pseudomonas spp.	2	18.4	26.0	0.04-36.8
Unknown Bacteria	2	0.4	0.3	0.20-0.7

**Genera sequenced that occurred in at least 33.3% (2 of 6) of the negative bone samples. The genera are sorted by the number of bone samples in which they were detected. Avg %, average percentage each genus contributed to its positive samples. SD, standard deviation of the percentages. Min-max %, range of the percentages. No hit, sequence has no match with the sequences in the NCBI database.*

Research and Testing Laboratory

PathoGENIUS™ Laboratories LLC

UT Southwestern Medical Center

1. Hartemann-Heurtier A, Senneville E (2008) Diabetic foot osteomyelitis. Diabetes Metab 34(2):87-95
2. Mutluoglu M, Sivrioglu AK, Eroglu M, et al. (2013) The implications of the presence of osteomyelitis on outcomes of infected diabetic foot wounds. Scand J Infect Dis 45(7):497-503
3. Citron DM, Goldstein EJC, Merriam V, et al. (2007) Bacteriology of moderate to severe diabetic foot infections and in vitro sctivity of antimicrobial agents. J Clin Microbiol 45:2819-28
4. CDCDFWC Consensus Development Conference on Diabetic Foot Wound Care (1999) Boston, Massachusetts. American Diabetes Association. Diabetes Care 22:1354-1360.
5. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D (2003) Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS ONE 3:e3326
6. Lipsky BA, Berendt AR, Cornia PB, et al. (2012) 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 54(12):e132-73
7. Senneville E, Melliez H, Beltrand E, et al. (2006) Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis 42(1):57-62
8. Quince C, Lanzen A, Davenport RJ, Turnbaugh PJ (2011) Removing Noise From Pyrosequenced Amplicons. BMC Bioinformatics 12:38
9. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10:996-998
10. Besman AN, Geiger PJ, Canawati H. Prevalence of corynebacteria in diabetic foot infections. Diabetes Care 1992;15:1531-1533
11. Goda A, Maruyama F, Michi Y, Nakagawa I, Harada K. Analysis of the factors affecting the formation of the microbiome associated with chronic osteomyelitis of the jaw. Clin Microbiol Infect 2014;20:309-317
12. Senneville E, Lombart A, Beltrand E, et al. Outcome of diabetic foot osteomyelitis treated nonsurgically: a retrospective cohort study. Diabetes Care 2008;31(4):637-42