P#2

Abstract Title:	**Molecular Analysis of Bacteria, Fungi and Antibiotic Resistance Genes in Diabetic Foot Ulcers**
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Objective:	Upon completion of the lecture, attendees should be better prepared to: ▪ Compare results of infected DFU wounds processed with molecular analysis to standard swab cultures. ▪ Identify a benefit of utilizing molecular analysis for identification of organisms in infected DFUs.
Abstract:	**Introduction:** Chronic wounds are a major healthcare problem in the United States with annual costs exceeding $10 million per year or half of total cost of all skin diseases. It is well-established that without control of infection, diabetic foot ulcers are difficult to manage and heal, despite proper glucose control, vascular optimization, off-loading and patient education. Often overlooked by the clinician is the complex interaction of not one primary microbe but multiple families of cooperating, DNA-sharing pathogens, protected by an extracellular polymeric substance (EPS), otherwise known as bio-film (Martin et al., 2010). While regular wound debridement to remove EPS has become standard of care, the on-going practice of culturing wounds, although inexpensive, to target infectious agents ignores those which cannot grow or divide slowly in culture media (selection bias) and misses organisms protected by EPS. Anaerobic testing not routinely performed in the initial patient work up can result in a failure to treat pathogens that have been shown to be present in up to a third of diabetic wounds (Dowd et al., 2008). Taking advantage of the unique 16S prokaryotic ribosomal subunit, molecular DNA-based polymerase chain reaction (PCR) and sequencing methods have proven to be reliable in targeting and demonstrating the presence of bacterial DNA found in wounds, revealing patterns of multiple coordinating bacteria as well as their resistance genes (Martin et al., 2010). **Methods:** This is a single-center, prospective study wherein swab specimens were compared using molecular analysis (GENETWORx, Glenn Allen, VA) of bacteria and antibiotic resistance to standard wound culture from suspected infection of diabetic foot ulcers. Samples that were positive for bacteria underwent sensitivity testing. Wound swab specimens were obtained using typical aerobic culture swab technique

and sent to the institution's microbiology lab while swabs for genetic analysis were sent out for independent processing to GENETWORx. Clinical decision making in prescribing antibiotic treatment was made on the basis of culture result and the patient's clinical condition and was not within scope of this study.

Results: Observed organisms from aerobic culture ranged from two to four species and were consistent with bacteria commonly isolated from diabetic foot ulcers at our institution. Except for Enterobacter and Proteus sp. (facultative) no anaerobic organisms were identified on culture media, while a variety of aerobic and anaerobic organisms were detected with molecular analysis. Total number of organisms isolated from molecular analysis ranged from three to fifteen species (mean= 6.8) per wound. There was general concordance with species type grown in culture compared to molecular analysis: All but one of the ten specimens identified a similar organism in both methods. Molecular analysis defined over 90% of all collected genetic material in each of the ten wounds tested and detected various common resistance genes, not demonstrated in culture specimens.

Conclusions: This study served as our institution's initial investigation of a technology that has the potential to obviate the need for the time-honored wound culture in treating diabetic foot ulcers. The use of molecular diagnosis to formulate targeted topical treatments has been demonstrated previously (Dowd, 2011), resulting in both cost-savings and time-to-healing.

This study supports a growing set of data that suggest that the current practice of selective systemic treatment of microorganisms that preferentially grow in vitro is likely inaccurate and ineffective, also increasing cost and healing time.

Molecular analysis allows the clinician a single, high-yield test that can reveal the characteristics of wound biomasses that are well-protected from systemic treatments by EPS and resistance genes to allow targeted local therapy.

The clinical application of molecular testing in the treatment of diabetic foot ulcers is developing; and results that reveal 2-3 times the number of active microbes, have the potential to complicate clinical decision making. Ignorance may be bliss, but in the care of chronic recalcitrant wounds, knowledge is power to the surgeon.

References and Resources:

1. Fife et al Wound Care Outcomes and Associated Cost Among Patients Treated in US O/P Wound Centers: Data From the US Wound Registry, Wounds 202; 24(1):10-17
2. Dowd SE et al, research survey of fungi and yeast in polymicrobial infections in chronic wounds J Wound Care 2011; 20:40
3. Wolcott, R. Economic aspects of biofilm-based wound care in diabetic foot ulcers. J Wound Care. 2015 May;24(5):189-90, 192-4. doi: 10.12968/jowc.2015.24.5.189

4. Wolcott, R, Cox, S.B. and Dowd, S.E. Healing and healing rates of chronic wounds in the age of molecular pathogen diagnostics. J Wound Care. 2010 Jul;19(7):272-8, 280-1
5. Martin, Jo, Zenilman, Jonathan and Lazarus, Gerald. Molecular Microbiology: New Dimensions for Cutaneous Biology and Wound Healing. 2010; 130: 38-48.

Disclosure:

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