Oral Presentation: Quantitative Analysis of PCR and DNA Sequencing of Chronic Wounds in the Philadelphia Area

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Materials and Methods

- Samples from 69 wounds from our clinic in Philadelphia, PA were analyzed by PCR and DNA sequencing analysis.
- The samples received real-time PCR analysis for “predominant bacteria” followed by DNA sequencing that screens for up to 25,000 known pathogens.
- 110 different species of bacteria were identified.
- On average, the sampled chronic wounds demonstrated 5.3 bacteria per wound.
- The most commonly identified major pathogens, i.e. bacteria representing 10 percent or more of the DNA in each sample, were: Corynebacterium striatum, Staphylococcus aureus, Pseudomonas aeruginosa, Alcaligenes faecalis, Staphylococcus lugdenensis, Proteus mirabilis, and Escherichia coli, respectively.
Statement of Purpose

The purpose of this study was to analyze the wound biome of the Philadelphia area using DNA sequencing in order to better understand the infections in our region and establish a more targeted therapeutic approach. Traditional methods of analyzing chronic wound flora only sample microorganisms that are easily grown on culture or in the specific database for real time PCR. By utilizing DNA sequencing, our aim was to demonstrate that the colonizing organisms vary from “the usual suspects.” In this study we compared the flora from chronic wounds using DNA sequencing results versus that of more traditional methods of testing, e.g. real time PCR.

Materials and Methods

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Procedure

We sampled 69 serial chronic wounds at Temple University Foot and Ankle Institute. All wounds were debrided of surface debris and slough using sterile gauze and sterile saline. A rough dry swab was used to collect as much surface material as possible.

Results

110 different species of bacteria were identified. On average, the sampled chronic wounds demonstrated 5.3 bacteria per wound. The most common major pathogens, i.e. bacteria representing 10 percent or more of the DNA in each sample, were as follows: Corynbacterium stratum, Staphylococcus aureus, Pseudomonas aeruginosa, Alcaligenes faecalis, Staphylococcus lugdenensis, Proteus mirabilis, and Escherichia coli, respectively. The most common wound colonizers were Staphylococcus aureus, Corynbacterium stratum, Staphylococcus lugdenensis, Finegoldia magna, Pseudomonas aeruginosa, Alcaligenes faecalis, Proteus mirabilis, Peptostreptobacter asaccharolyticus, Anaerococcus vaginalis, Streptococcus agalactiae, Escherichia coli, and Staphylococcus epidermidis, respectively.

Figure 1. Most Common Pathogens of Chronic Wounds in Philadelphia. Major pathogen is defined by representing 10% or more of the DNA found in the wound

Figure 2. Most common wound colonizers

Literature Review

Traditional wound cultures rely on isolation on agar and physical identification of micro-organisms that survive transport and are able to grow in the lab environment. Only 1% of bacteria cultured can be grown easily in most hospital laboratories. Clinicians have recognized these bacteria as the infecting organisms in most wounds; however, these are just the organisms that are easiest to culture. Clinical training leads us to believe that infections are caused by one major pathogen based on standard culturing techniques; clinical tests we use are optimized to culture planktonic bacteria rather than biofilms. It has been demonstrated that 60-90% of chronic wounds have bacteria existing in biofilms. Biofilms require specialized culturing techniques, real time PCR, or DNA sequencing analysis. Panbacterial real-time PCR is an effective method of determining the total bacterial load in chronic wounds based on a predetermined set of primers, and DNA sequencing from a library of thousands of known microorganisms. Bacterial communities are specific to the local environment and PCR/DNA testing gives an expanded view of organisms in the local biome. It has been demonstrated in previous regional studies that epidemiological monitoring of bacterial species in the local wound biome and analysis of antibiotic sensitivity profiles play an essential role in developing an antibiotic resistance policy and an effective therapeutic strategy.

Analysis and Discussion

In our sample set, the most prevalent colonizing organism was Staphylococcus aureus, followed by Corynbacterium stratum, Staphylococcus lugdenensis, and E. coli, respectively (Figure 2). However, the most common colonizing organism does not tell the entire story since most of the wounds evaluated in this study had bacteria existing in biofilms. On average, the sampled wounds contained 5.3 different species of bacteria per wound. This biofilm must be addressed when considering therapeutic strategy. The most ubiquitous major pathogen in our sample set, meaning 10% or more of the total DNA found in the wound, was Corynbacterium stratum (17%), followed by Staphylococcus aureus (16%), and Pseudomonas aeruginosa (14%) (Figure 1). A major pathogen is defined by representing 10% or more of the DNA found in the wound. Our study demonstrates that the most common major pathogen was not the expected “Staph or Strep” species, but Corynbacterium stratum. C. stratum, even though frequently isolated has been overlooked in the treatment of chronic wounds and it’s role is not well understood. Although C. stratum is often dismissed as non-pathogenic, Patel S. et al. demonstrated four cases of C. stratum osteomyelitis in the Greater Philadelphia area in patient’s with non-healing diabetic foot ulcers and Diphtheroids on bone culture. Until we know the predominant pathogen is present in our immediate environment, we cannot effectively set PCR primers for more cost effective and accurate PCR testing.
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Conclusion

• The most common colonizing organism does not tell the entire story since most of the wounds evaluated in this study had bacteria existing in biofilms.

• On average, the sampled wounds contained 5.3 different species of bacteria per wound. This biofilm must be addressed when considering therapeutic strategy.

• Standard culture techniques are optimized to culture planktonic bacteria rather than biofilms.

• It has been demonstrated that 60-90% of chronic wounds have bacteria existing in biofilms.

• PCR/DNA testing gives an expanded view of organisms in the local biome.