JUMPSTARTING CHRONIC WOUND HEALING USING TOPICAL ANTIBIOTIC COMPOUNDS INFORMED BY NGS MICROBIAL PROFILING: A SINGLE CENTER RETROSPECTIVE ANALYSIS IN PATIENTS WITH MIXED ETIOLOGY PERSISTENT WOUNDS

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Abstract

Background - Chronic wound healing remains a significant challenge and up to 78% of chronic wounds are estimated to contain biofilm which delays healing. Biofilm-based wound care hypothesizes that microbial burden must be managed for wounds to heal and avoid the worst complications of a chronic wound. Prior work has established that disrupting the wound microbiome may encourage healing, including the use of topical antibiotics. The primary objective of this retrospective analysis was to quantify the healing rates of difficult-to-heal wounds with an applied topical antibiotic compound based on the microbiome, quantified by next generation sequencing (NGS).

Methods - A single-center retrospective chart review was performed to quantify the healing rates of chronic wounds treated with topical antibiotic lipid-based compounds informed by NGS. All patients receiving NGS guided topical antibiotics failed to show improvement after at least one other intervention, including topical antiseptics, topical antibiotics, and other wound products. Other standard practices such as debridement, compression, off-loading, nutritional support, and arterial and venous optimization followed institutional guidelines. Wounds were swabbed for microbial profiling through a validated Laboratory Developed Test service and then started on a topical antibiotic mixed with lipogel selected based on the microbial results. Wound area measurements were compared at 4-week intervals from approximately 8 weeks prior to starting treatment through 20 weeks after. Percent area reduction (PAR) was calculated using the treatment start date as the reference measurement. Baseline microbial profiles were compared against 20-week outcomes.

Results - Following chart review, fifteen patients with 19 unique wounds were identified who started the NGS guided topical antibiotic treatment between July 2021 and January 2024. Wounds were mixed etiology, with venous stasis ulcers (74%) being the most common and were present for 2.1 years on average (Q1=0.6 years, Q3= 3.0 yrs) prior to initiating NGS guided therapy. By 20 weeks, 21% of wounds achieved complete healing and 84% had greater than 60% PAR, though significant PAR was observed as early as 4 weeks ($p<0.001$). There was no consistent change in wound PAR prior to starting NGS guided therapy ($p>0.05$). *Staphylococcus aureus* was overrepresented among wounds healing within 20 weeks, whereas persisting wounds were more diverse with greater abundance of *Pseudomonas* and *Corynebacterium*.

Conclusion - Most patients in this retrospective series saw significant improvement or healed completely using a topical NGS-guided antibiotic regimen, consistent with prior literature. Our retrospective analysis supports the use of topical antibiotic treatment informed by molecular diagnostics to heal persistent nonhealing wounds and provides valuable preliminary data for use in planning a more stringent randomized controlled trial.

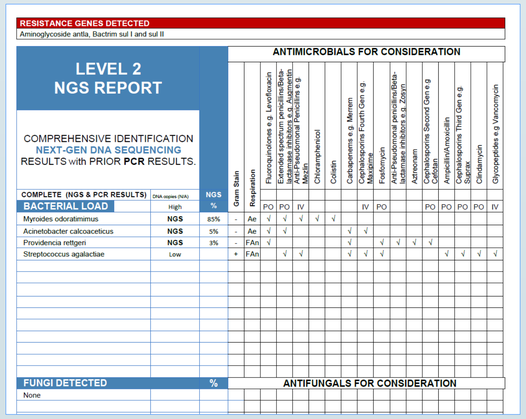
Introduction

- Many chronic wounds do not heal despite following best practice wound care management.
- Up to 78%[1] of chronic wounds have a biofilm and microbial growth that likely delays wound healing.
- Debridement is part of the treatment for biofilms, but this complex matrix will quickly regrow within 24 hours post debridement and is not enough to eradicate a biofilm.
- Additionally, many broad-spectrum antiseptic wound products do not eliminate microbial burden enough to allow the wound to heal.
- Targeted microbial profiling by Next Generation Sequencing (NGS) improves detection of microbial burden in the wound bed compared to culture alone[2].
- Lipogel is an ideal base for topical antibiotics as lipogel is a surfactant, improving disruption of bacterial bonds and improving penetration of the topical antimicrobial into the wound bed[3].
- Topical antibiotic compounds guided by NGS may improve healing of chronic wounds[4], especially those not responding to standard wound care.

Methodology

A retrospective chart review was performed for patients receiving wound care at Corewell Health West. Patients with recalcitrant wounds who received topical compounded antibiotics guided by NGS were selected for inclusion. Upon selection, wound dimension measurements were recorded in 4-week intervals from approximately 8 weeks prior to starting treatment through 20 weeks after.

- Samples were taken after debridement of the wound bed. If tissue was available after debridement, this was collected and sent in a specimen tube. The wound was then swabbed in its entirety at the wound bed for sample.
- Collected specimens were shipped overnight and analyzed per the WoundKey test service (MicroGen DX, Lubbock, TX). The WoundKey NGS test includes comprehensive bacterial 16s rRNA gene and fungal ITS sequencing, as well as a limited qPCR panel for rapid reporting of common wound microbes and antibiotic resistance associated genes (protocol detailed here[5]).
- After receiving the NGS report (example report shown Figure 1), a custom topical antibiotic regimen mixed in lipogel was made and used on the wound bed daily. Moisture balance was optimized with secondary dressings as needed and gauze.
- Wound area and wound volume were calculated based on width, length and depth measurements, then transformed to percent wound area (PWA) or percent wound (PWV). The wound measurements recorded at sampling for NGS was considered the starting point of therapy and used as the reference for comparison. Pairwise comparisons between each time point were analyzed by wilcox test.
- Baseline bacterial profiles were compared against 20-week outcomes.



RESISTANCE GENES DETECTED

Aminoglycoside antia, Bactrim sul I and sul II

LEVEL 2 NGS REPORT

COMPREHENSIVE IDENTIFICATION NEXT-GEN DNA SEQUENCING RESULTS with PRIOR PCR RESULTS.

ANTIMICROBIALS FOR CONSIDERATION

COMPLETE (NGS & PCR RESULTS)	DNA copies (N/A)	NGS	Gram Stain	Respiration	Fluoroquinolones e.g. Levofloxacin	Fluoroquinolones e.g. Ciprofloxacin	Inhibitors (Inhibitors e.g. Augmentin	Anti-Pseudomonal Penicillins e.g. Zosyn	Chloramphenicol	Colistin	Carbapenems e.g. Meropen	Cephalosporins Fourth Gen e.g. Maxiatime	Fosfomycin	Aminoglycosides (penicillin-Beta-lactamase inhibitors e.g. Zosyn)	Aztreonam	Cephalosporins Second Gen e.g. Cefotetan	Ampicillin/Amoxicillin	Cephalosporins Third Gen e.g. Suprax	Clindamycin	Glycopeptides e.g. Vancomycin
BACTERIAL LOAD	High	%			PO	PO	IV				IV	PO				PO	PO	PO	PO	IV
Myroides odoratimimus	NGS	85%	-	Ae	√	√	√	√	√											
Acinetobacter calcoaceticus	NGS	5%	-	Ae	√	√					√	√								
Providencia rettgeri	NGS	3%	-	FAn	√						√		√	√	√	√				
Streptococcus agalactiae	Low		+	FAn		√	√				√	√	√				√	√	√	√

FUNGI DETECTED %

None

ANTIFUNGALS FOR CONSIDERATION

Figure 1. Example NGS microbial report provided by MicroGen DX.

Results

We present on 18 distinct wounds in 15 patients that were resistant to multiple prior treatment modalities. Wounds had all been considered to fail at least one prior antimicrobial intervention and were present for 2.1 years on average (Q1=0.6 yrs, Q3=3.0 yrs). Venous leg ulcers were the most common etiology (74%).

Key finding #1 - Significant reductions in wound area and volume (Fig 2).

- The mean PWA at 4 weeks was 68.4% of the starting wound area ($p<0.0001$) or PWV at 61.0% ($p<0.001$).
- Further significant PWA reductions from week 4 through weeks 12-20 ($p<0.05$).
- At 20 weeks, 21% of wounds were completely healed.

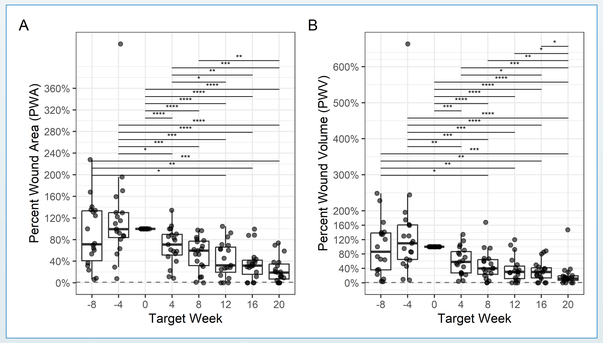


Figure 2. (A) Percent wound area and (B) percent wound volume over time measurements show significant decreases over time. Week '0' indicates the week that NGS guided topical antibiotics were started and was when a baseline measurement was taken to calculate PWA and PWV for other time points. The week number shown indicates the target week for wound measurement and may deviate by a week (average standard deviation across all target weeks = 1.0 week). Significance asterisks indicate non-adjusted p-values: '.'=0.05<p<0.10; '*'=0.01<p<0.05; '**'= 0.001<p<0.01; '***'= 0.0001<p<0.001; and '****'= p<0.0001.

Key finding #2 – Greatest improvement rates through 8 weeks

- No significant PWA reductions were noted from 12 weeks to later time points (Fig 2), though mean PWA decreased from 43% in wk 12 to 23.4% in wk 20.
- Qualitatively, the healing rate appears to slow and there may be an 'elbow' present at 6 wks where returns begin to diminish. (Fig 3)

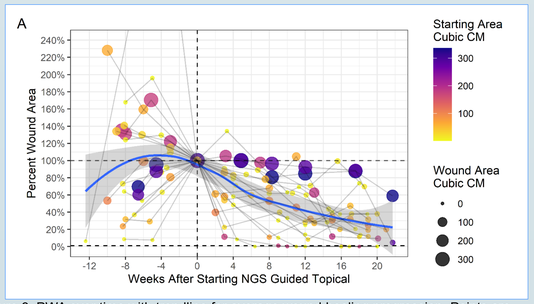


Figure 3. PWA over time with trendline for average wound healing progression. Points are sized by area per visit, colored by size at baseline measurement, and repeated measurements of wounds are connected by grey lines. Horizontal dashed lines indicate 0% and 100% measurement lines from baseline. A vertical line indicates the reference measurement.

Results (continued)

Key finding #3 – Paradoxes between baseline microbiota and 20-wk healing outcomes (Fig 4).

- Low diversity and abundant Staphylococcus in wounds which healed by 20 weeks.
- Conversely, wound microbiota shared similarities between the worst and best healing wounds.
- Worst healing wounds were generally more abundant in Staphylococcus AND Corynebacteria.

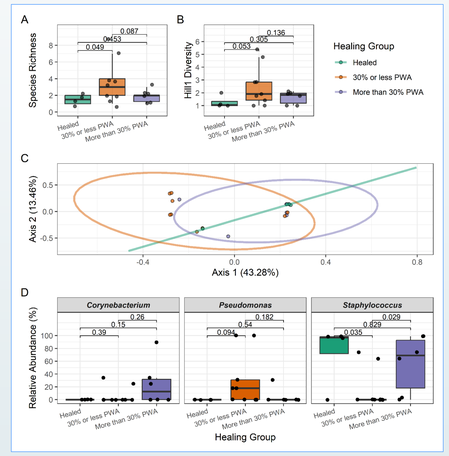


Figure 4. The bacterial composition of wounds at baseline identified by 16s rRNA gene sequencing used to guide antibiotic choice, shown per (a) individual wound or (b) aggregated according to 20 week healing outcome. Healing outcome groups were defined based on their percent wound area (PWA) from the time NGS guided topical treatment was initiated.

Conclusion

NGS guided topical antibiotic treatment are effective for patients that have failed other antimicrobial options and wounds have failed to improve or worsened with standard wound therapy.

The current results suggest that wounds should show improvement within 4-6 weeks.

If a wound initially shows improvement with NGS guided topical antibiotics, but then stalls or worsens again, the wound may need to be re-tested a new compound formulated to target the shifting microbial profile.

References

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