# Association of Aging, Frailty and Place of Residence with Skin, Oral and Gut Microbiome Characteristics and Pathogenicity Reservoirs

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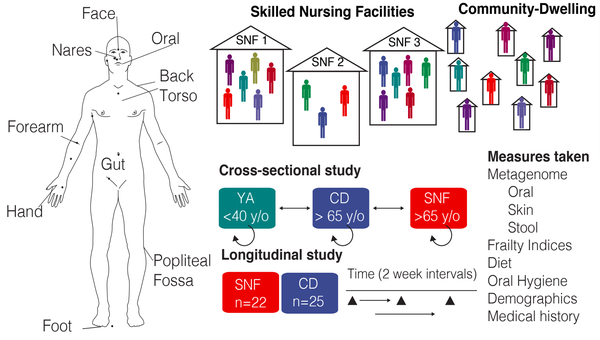
## Introduction

Despite their elevated risk for morbidity and mortality from infections, the microbiome of older adults remains understudied. While colonization resistance from resident microflora is a promising means to prevent infections, little is known about pathogenicity reservoirs and colonization resistance in this vulnerable population. We studied the skin, oral, and gut microbiome dynamics of older adults in both community and Skilled Nursing Facility (SNF) settings, investigating relationships between age, frailty, environment, microbiota, and pathogenicity reservoirs.

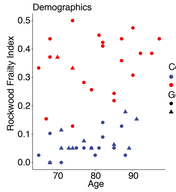






| Demographic Characteristics | CD | SNF |
|---|---|---|
| Total No. | 25 | 22 |
| Age range, y | 65-91 | 65-97 |
| Age, y, Mean (SD) | 78.2(7.59) | 82.9 (8.46) |
| Sex, No. (Self-Reported) | | |
| Male Participant | 11 | 2 |
| Female Participant | 14 | 20 |
| Race | | |
| American Indian or Alaska Native | 1 | 0 |
| White | 24 | 20 |
| More than one race | 0 | 2 |
| Ethnicity | | |
| Hispanic or Latino | 1 | 2 |
| Non-Hispanic or Latino | 24 | 18 |
| Height, mean (SD), cm | 167 (11.9) | 160 (8.5) |
| Weight, mean (SD), kg | 69.0 (15.4) | 78.7 (23.9) |

**Measures taken**
Metagenome
Oral
Skin
Stool
Frailty Indices
Diet
Oral Hygiene
Demographics
Medical history




We conducted a longitudinal metagenomic whole genome shotgun survey of 47 adults age 65+ years of age; 22 residents of 3 different SNFs and 25 community dwelling individuals. We performed metagenomic whole genome shotgun sequencing on stool, oral, and skin samples from 8 sites, 1421 total. To correlate clinical and behavioral variables, we measured frailty, collected medical records, and interviewed participants on diet and lifestyle. We also draw comparisons with previous younger cohorts[1-3].

## Results

### Instability, Heterogeneity, Hyperdiversigication, and Anarchy in the Aging Skin Microbiome

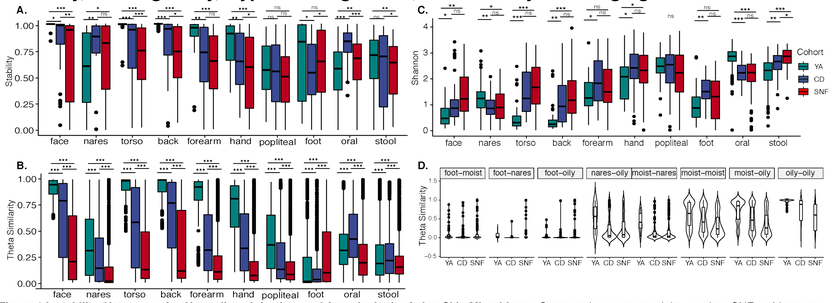



**Figure 1 Instability, Heterogeneity, Hyperdiversigication, and Anarchy in the Aging Skin Microbiome.** Compared to younger adults, or when SNF residents are compared to Community-Dwelling older adults the taxonomic composition of the skin microbiota was generally characterized by:

A. Decreased stability over time. Yue-Clayton Theta Index comparing samples from an individual at different timepoints. Where most younger adult skin sites are relatively constant overtime, the skin microbiota of older and frailer adults appears to vary substantially.

B. Decreased inter-individual similarity. Yue-Clayton Theta Index comparing samples between individuals in each cohort. Older and frailer adults exhibited far less skin microbiome similarity to their peers than younger adults, demonstrating higher Heterogeneity.

C. Hyper-diversification (Shannon Index of diversity representing the number and evenness of species). This trend was also observed in the gut.

D. Decreased Intra-individual heterogeneity. Yue-Clayton Theta Index comparing samples from different skin sites on the same individual at the same timepoint. Rather than becoming more similar with skin aging, skin sites appear to diverge in the older and frailer cohorts. Since this is combined with instability and inter-individual divergence thus representing a breakdown in order, rather than a transition to a new one, we refer to this phenomenon as anarchy.

CD=Community-Dwelling; SNF= Skilled Nursing Facility; YA=Younger Adults. Bidirectional Wilcox tests, *=p<0.05, **=p< 0.0005, ***=p<5E-8.

### Taxonomic Compositional Differences in the Microbiota of Older Adults.

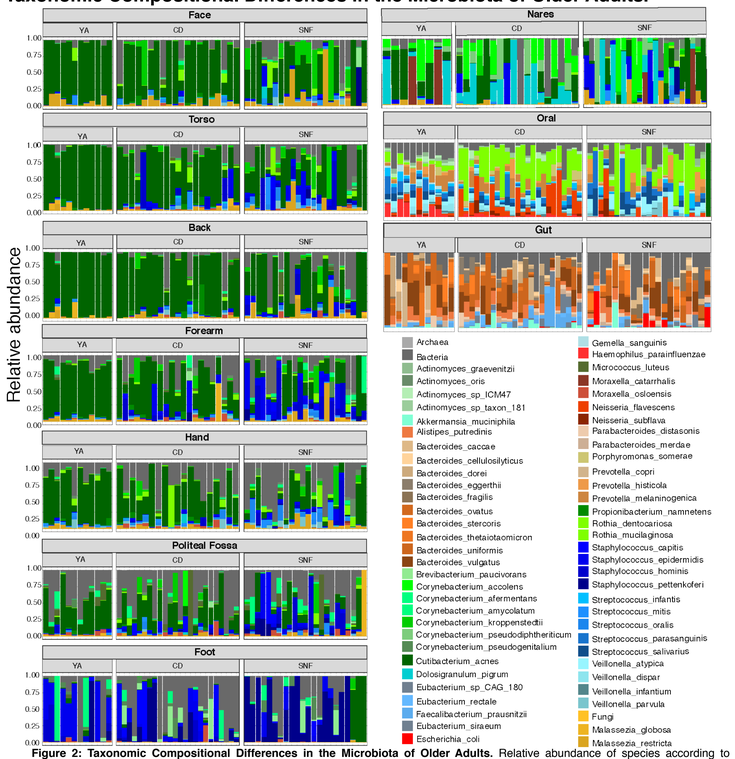



**Figure 2: Taxonomic Compositional Differences in the Microbiota of Older Adults.** Relative abundance of species according to MetaPhlAn 3.0[4] classification. Each bar represents 1 subject, 1 timepoint represented per subject. Older adults, especially SNF residents, exhibit marked decrease in cutaneous *Cutibacterium acnes* abundance, with a reciprocal increase in Staphylococci, Corynebacteria, and in some cases *Malassezia* and oral species. High inter-individual heterogeneity in older cohorts is also evident here. Oral (tongue dorsum) had notably higher abundance of *Rothia* species, and notably less Proteobacteria in the SNF cohort. Gut microbiota of SNF residents had a higher Firmicutes:Bacteroides Ratio, and in many cases increased Proteobacteria and decreased *Akkermansia muciniphila*.

### Associations Between Age, Frailty, and Structural Differences in the Skin Microbiome

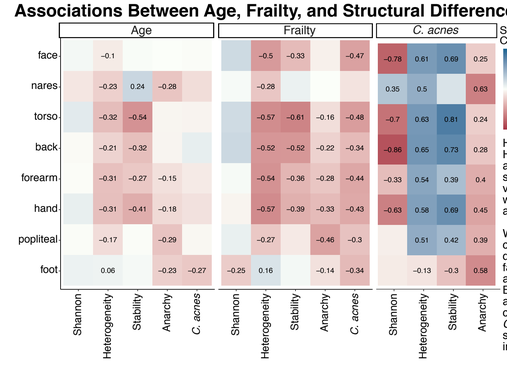



**Figure 3: Associations Between Age, Frailty, and Structural Differences in the Skin Microbiome.** Mixed Effects Model controlling for temporal pseudoreplication testing linear correlation by skin site between age, frailty (Rockwood Index), or *C. acnes* abundance and Shannon Diversity Index, Inter-individual Heterogeneity, Stability, or Intra-individual Heterogeneity (Anarchy). Only significant (fdr adjusted $p < 0.05$) Spearman's Coefficients shown. Age was a poor predictor of all variables. Frailty was negatively correlated with *C. acnes* abundance and heterogeneity across skin sites.

We hypothesized that skin aging, characterized by follicular atrophy and decreased sebum production, creates a less favorable environment for *C. acnes* growth, and that the resulting decrease is correlated. Because the Rockowrd Frailty Index is an aggregate score not directly indicating skin condition, we tested the relationship between *C. acnes* and the other variables, finding strong correlations at nearly all skin sites, indicating a pattern of dysbiosis.

### Skin a Reservoir for Specific Pathobionts in Older Adults

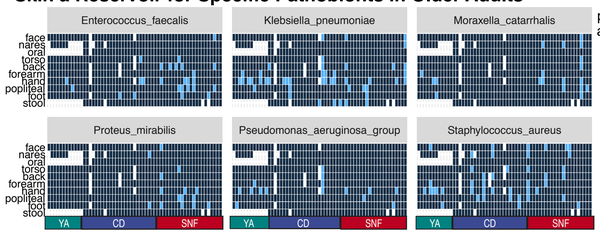



**Figure 4: Skin a Reservoir for Specific Pathobionts.** Presence/Absence heatmap of specific pathobionts identified with metagenomic shotgun sequencing. This method is less sensitive for low-abundance pathogens than culture-based screening, but still found clinically significant pathobiont colonization in the skin more frequently than oral or gut.

### Skin Major Reservoir of Plasmid Anti-Microbial Resistance in Older Adults

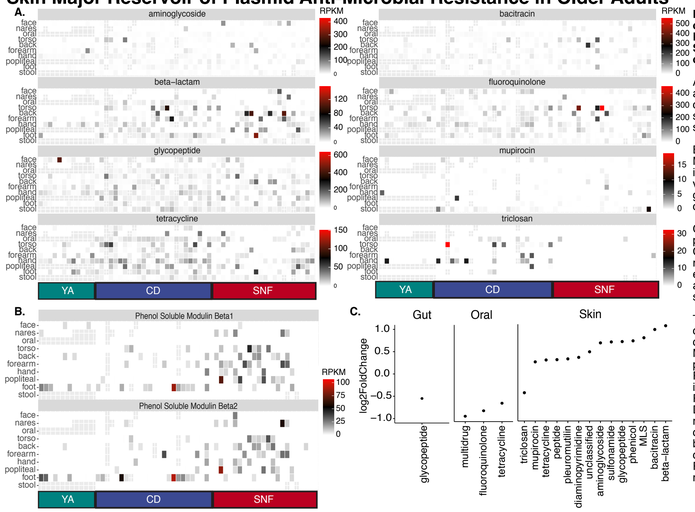



**Figure 5: Skin Major Reservoir of Plasmid Anti-Microbial Resistance and Staphylococcal virulence in older adults.**

A) Abundance of Plasmid antimicrobial resistance (AMR) class. Each column represents a subject. One timepoint per subject.

B) Abundance of Phenol Soluble Modulin (PSM) genes. PSMs are important Staphylococcal virulence factors, which we found greatly enriched in the SNF cohort.

C) Differential Abundance of plasmid AMR classes between CD and SNF cohorts. SNF resident skin microbiome exhibited significantly higher abundance of many clinically significant AMR classes.

To identify plasmid ARGs, contigs generated from quality-controlled reads using MEGAHIT were classified as plasmid or genomic using Plasflow. Plasmid genes were identified from contigs with Prodigal, and finally mapped to DeepARG[5,6]. Samples RPKM normalized to account for differences in sequencing depth between samples and differences in target gene length. RPKM=Reads per kilobase million.

## Summary

We conducted a novel, longitudinal, gut, oral, and skin metagenomic whole genome shotgun study of older adults both in skilled nursing facilities and living privately in the greater community. To the best of our knowledge, this is also the largest report to date of the skin metagenome in older adults.

We found that in particular the skin microbiota of older adults are substantially different to those of younger adults. In particular, we found:

- Major compositional differences between healthy older adults and younger adults, as well as SNF residents to Community-Dwelling older adults including:
  - Decreased relative abundance of *C. acnes*
  - Increased Staphylococci, Corynebacteria, fungi, and oral species
- Substantially decreased stability of the skin microbiota
- High inter-individual heterogeneity
- High intra-individual heterogeneity
- Age alone is a poor predictor of these changes.
- There are strong correlations between decreased *C. acnes* abundance and instability, hyper-diversification, and hyper-heterogeneity. This indicates a pattern of dysbiosis.
- The skin microbiome in older adults, and particularly SNF residents, serves as a major reservoir of clinically important pathobionts and antimicrobial resistance.

## Conclusions

Although preliminary, we believe that these results represent foundational findings in our understanding of the microbiota of older adults. In particular, they demonstrate dramatic differences in the skin microbiome among older adults. We suspect that skin aging is a key driver in these changes, adversely affecting C. acnes and leading to a breakdown in community structure, although this possibility cannot be directly addressed by our dataset and must be a subject of future research. Most importantly, our findings draw attention to the skin as potentially a more important reservoir than the oral and gut microbiota for clinically relevant pathogens and antimicrobial resistance.

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