

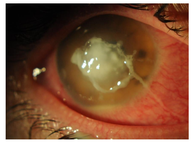
The Corneal Microbiome in Health and Microbial Keratitis: New Insights from Shotgun Metagenomic Data

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Background



Little is known about the corneal microbiome and what constitutes a pathogen in microbial keratitis (MK).

We carried out a prospective observational study to improve understanding of the corneal microbiome in health and disease.

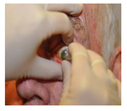
Methods

1. Participant recruitment: 146 MK patients and 18 healthy controls

2. Sample collection: x2 corneal impression membranes (CIMs) collected from:

- **Affected-eye** of MK participants
- **Non-affected eye** of MK participants
- One eye of **healthy control** participants
- **Environmental controls** – "air" CIMs collected at time of participant sampling





3. Sample processing:

- **CIM 1:** Conventional diagnostic culture.
- **CIM 2:** Shotgun metagenomic sequencing (Illumina).

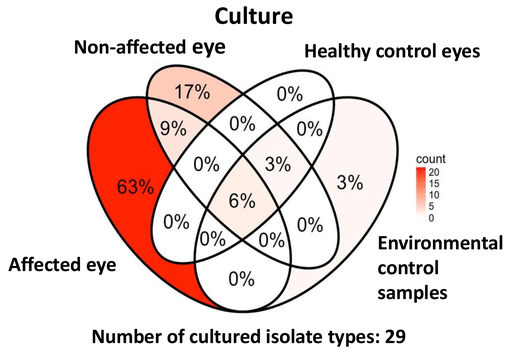
4. Bioinformatical analysis:

- **Quality control:** FastQC/MetaWRAP
- **Taxonomy on reads**: Kraken2 (customised reference database)
- **Metagenomic assembly/binning:** Metaspades/MetaWRAP
- **Positive shotgun metagenomic result:** microbe >20% relative abundance

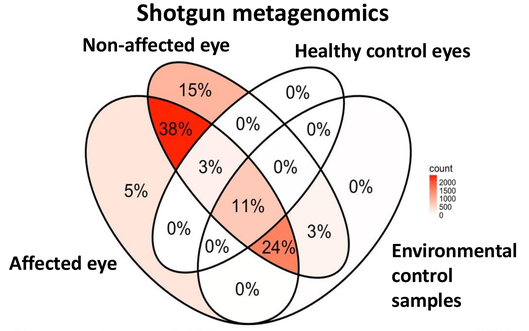
Results

1. Does the microbiome of MK-affected eyes differ from non-affected eyes and healthy control eyes?

i. Microbial overlap between MK-affected eyes, non-affected eyes, and healthy controls

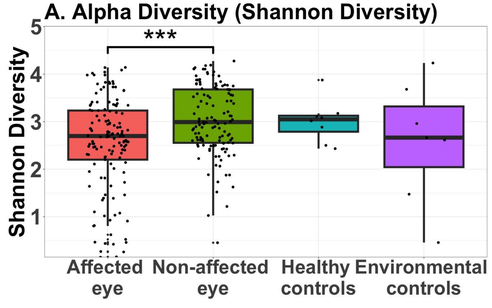


Number of cultured isolate types: 29



Number of taxa = 6,621 operational taxonomic units (OTUs)

ii. Microbial diversity differences by sampling site



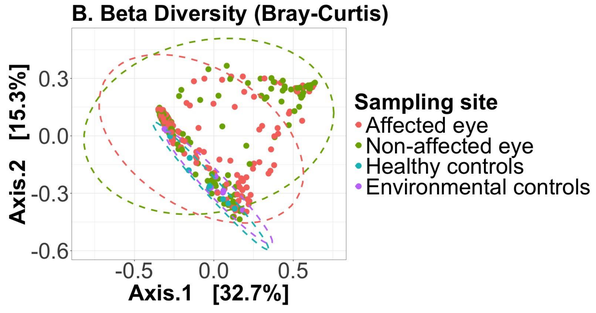


Figure 1. A) Alpha diversity (Shannon diversity) was significantly lower in affected eyes compared to non-affected eyes (***p < 0.001, ANOVA with Tukey's post hoc test), **consistent with microbial dominance by a single organism.** No significant differences were observed between any other groups. B) Principal coordinates analysis (PCoA) of Bray-Curtis dissimilarity shows distinct similarity in microbial composition. Affected eyes differed significantly from all other groups (p < 0.01, PERMANOVA, 999 permutations), while healthy and environmental controls did not differ (p = 0.321). **Notably, non-affected eyes differed from healthy controls (p = 0.002), suggesting the presence of an altered or "at-risk" corneal microbiome in patients with MK.**

2. How can shotgun metagenomics improve MK diagnosis?

i. Culture vs shotgun metagenomics for MK pathogen identification

		Culture		
		MK pathogen detected	No pathogen detected	Total
Shotgun	MK pathogen detected	19	16	**35 (24.0%)**
	No pathogen detected	17	94	**111 (76.0%)**
	Total	**36 (24.7%)**	**110 (75.3%)**	**146**

Table 1: Comparison of MK pathogen detection by culture and shotgun metagenomics. Chi-squared test, p < 0.01. **Combining culture and shotgun metagenomics increased pathogen detection to 35.6%, significantly higher than either method alone (p < 0.001).**

ii. Metagenomic assembled genomes from MK-affected eyes

Pseudomonas aeruginosa from affected eye (one example):

- Genome fraction 90.6%
- 211 virulence genes identified
- 46 resistance genes identified

Discussion and conclusions

- First study to identify a corneal microbiome using shotgun metagenomics.
- Shotgun metagenomics reveals that the non-affected eyes of MK patients harbor a distinct microbiome, differing from both affected eyes and healthy controls, suggesting the presence of an at-risk corneal microbiome that may predispose to disease.
- Culture and shotgun metagenomics exhibit partial diagnostic concordance, with each method detecting unique pathogens; their combined use enhances diagnostic sensitivity, and shotgun further provides insights into microbial virulence factors.