

AIM

Investigate the difference in relative abundance of human pathogens found in metagenome samples from kitchen drains and bathroom drains.

BACKGROUND

Presence of pathogenic species in common households is a cause for concern for human health.

Recent incidents of localized *Legionella spp.* in Copenhagen.¹

Pathogens e.g. *Legionella spp.*, *Salmonella spp.*, *Campylobacter spp.* and *Pseudomonas spp.* have been identified in common household kitchens and bathrooms.²⁻⁴

Kitchens are found to be more contaminated.²⁻⁴

HYPOTHESIS

“There is no difference in relative abundance of pathogens between kitchens and bathrooms.”

ANALYSIS

Figure 1: Sequencing evaluation

Nonpareil curves represent fitted sigmoidal functions based on the estimated average coverages (empty circles) per sample. Projections suggest the required amount of sequencing (bp) to cover a near-total diversity.

Figure 2: Sample composition overview

An overview of the composition ratios for all samples by pathogenic or non-pathogenic bacteria classification. Unclassified organisms could not be mapped to bacteria, viruses or archaea.

Figure 3 & 4: Sample abundance profiles

The box plots for bathroom and kitchen samples show high similarity in composition. The slight negative skews in some of the plots are most likely related to the different numbers of classified species in some of the samples as seen in figure 2.

Figure 5: Shannon Index

Per sample Shannon indices of pathogenic species. Higher values of the Shannon index indicates the presence of many species with equal abundance within the sample.

Figure 6: Bray-Curtis dissimilarity

The Bray-Curtis dissimilarity index is bounded between 0 and 1, where 0 means the two sites share sample composition perfectly, whereas 1 indicates a 100% dissimilar sample composition.

Figure 7: Robust PCA

As is shown in the plot, no clear division of kitchen and bathroom samples is visible in the first two principal components.

Figure 8: Heatmap

Clustering hierarchically column-wise yielded only minor aggregates of clusters. Largest cluster observed consists of 4 bathroom samples.

CONCLUSION

- The null-hypothesis of no difference in relative abundance of human pathogens between kitchen and bathroom facilities cannot be rejected. Furthermore, the difference in relative abundance between individual species in kitchen vs bathroom samples could not be detected in the 465 species found in the samples.
- The most frequent pathogens in the samples (*Pseudomonas*, *Stenotrophomonas*, *Paracoccus*, *Moraxella*) can be responsible for infections of the respiratory tract, urinary tract, eye, ear, skin but mostly in immunocompromised individuals.¹⁰⁻¹³
- Future perspectives: The sequencing depth could be increased to cover more species. More kitchen samples are needed. Metadata could be used to analyze the possible reasons for the differences in microbial composition between samples.

WORKFLOW

1. SAMPLE COLLECTION

Sampling
Purification
Illumina sequencing

2. QUALITY PROCESSING

QC using Multiqc⁵
Cutadapt⁶:
Adapter removal
Bias removal (3 bp)
Quality trimming < 20
Length filtering < 30 bp

Super deduper⁷:
Deduplication

3. TAXON MAPPING

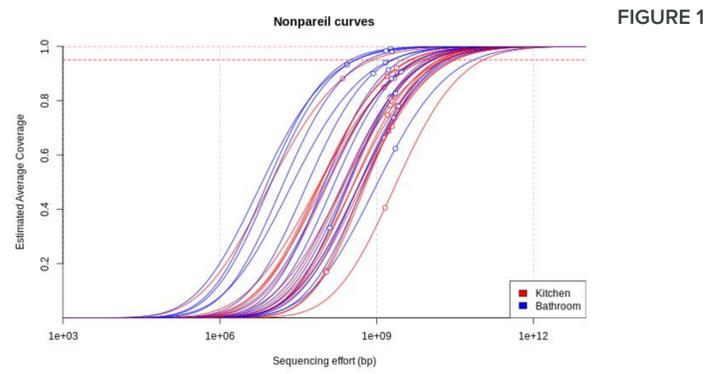
Kaiju⁸
PATRIC⁹

4. ANALYSIS

Composition
Nonpareil curves
Shannon-Index
Bray-Curtis dissimilarity
Wilcoxon signed rank test
Heatmaps

5. CONCLUSION

SEQUENCING EVALUATION



SAMPLE PROFILE

Composition by classification

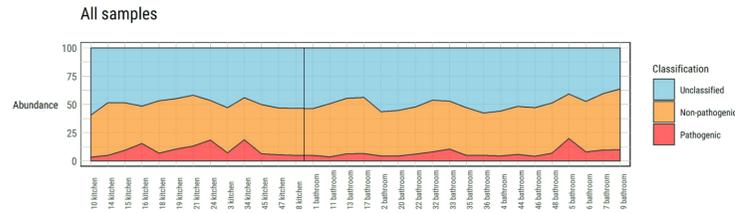
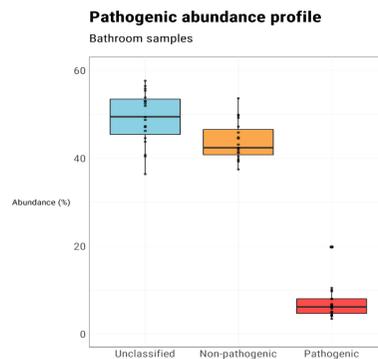
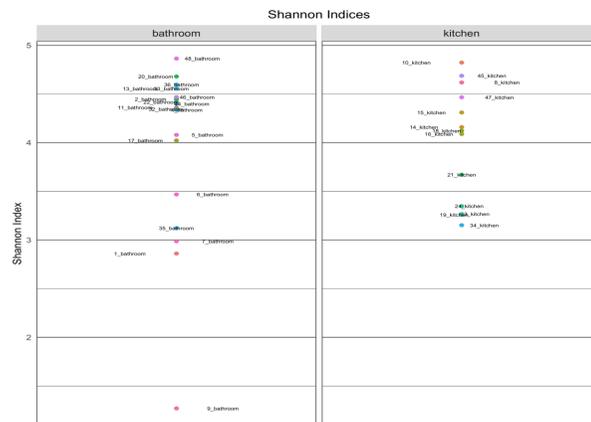


Figure 3



DIVERSITY

Figure 5



CLUSTERING

Figure 7

Robust PCA of Bathroom vs. kitchen

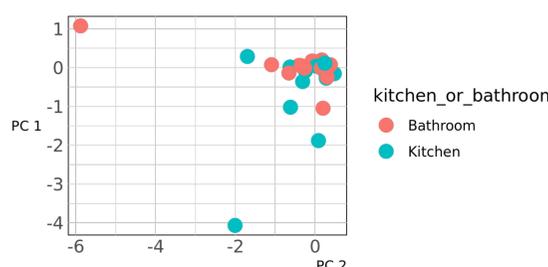
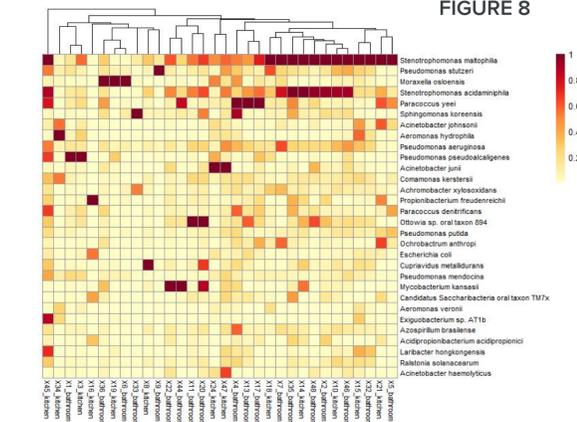


Figure 8



Key values:	Unclassified reads*	Non-Pathogens	Pathogens	Average Shannon-Index	Average Coverage	H1 (Wilcoxon) p-value
Bathroom:	49.49%	44.06%	6.44%	3.991	60.68%	0.1578
Kitchen:	49.06%	41.63%	9.3%	3.997	72.48%	

* Could not be mapped to bacteria, viruses or archaea

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[5] Philip Ewels, Måns Magnusson, Sverker Lundin and Max Källner, "MultiQC: Summarize analysis results for multiple tools and samples in a single report" doi: 10.1093/bioinformatics/btw354

[6] Marcel Martin, "Cutadapt removes adapter sequences from high-throughput sequencing reads.", DOI: 10.14806/ej.171.200

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