Oral Paleomicrobiome of a Medieval/Early Modern Portuguese Community

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INTRODUCTION

Human skeletal remains and the burial context in which they are exhumed are valuable sources of information about the lifestyle, health, disease, diet, and environment of past populations.

Dental calculus has emerged as a **key** material in the study of past health and microbiome. It forms on the surface of teeth from microbial biofilms and food debris that are not removed and eventually become mineralized.

The oral cavity hosts the **oral microbiome**, the second largest microbial community in the human body after the gastrointestinal tract. An imbalance in this microbial ecosystem—known as **dysbiosis**—can lead to the proliferation of pathogenic bacteria, resulting in oral diseases such as caries, gingivitis, and periodontitis.

This study focuses on individuals from the Nossa Senhora da Salvação churchyard in Arruda dos Vinhos (14^{th}-16^{th} centuries).

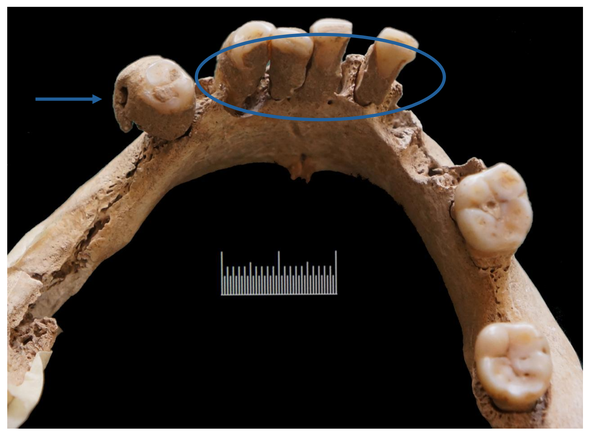


Figure 1: Mandibular teeth with calculus from individual no. 35.

Figure 2: Interproximal view of teeth 26 and 27 with calculus from individual no. 35.

Figure 3: Burial of individual no. 35 in the churchyard of the Igreja de Nossa Senhora da Salvação.

AIMS

This study aims to analyse the oral microbiome of adult individuals exhumed from a necropolis of the 14th–16th centuries, to better understand the microorganisms present in the oral cavity during this period. The study also seeks to infer oral pathologies and dietary habits of these individuals.

MATERIALS & METHODS

Samples selection:

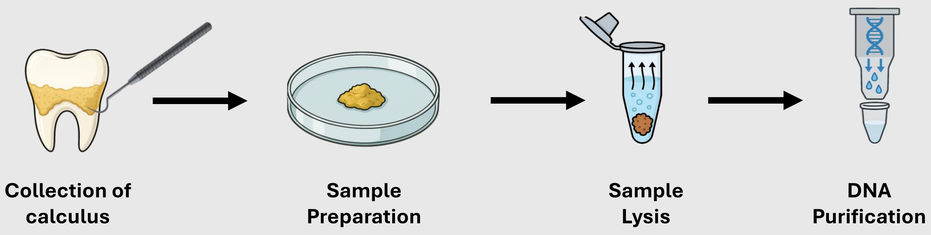
80 individuals exumed → **41** adults → **29** individuals with **skull** → **23** individuals with **teeth** → **14 with calculus**

Anthropological analysis:

- Sex – Bones analysis.
- Age at death – 2 methods → **Suchey-Books** + **Buckberry and Chamberlain**.
- Stature – **Femur** and **Humerus** length.
- Oral pathologies: caries, occlusal wear, Horizontal Linear Enamel Hypoplasia (HLEH), periodontal disease, periapical inflamations and calculus.

Laboratory procedures:

- Calculus collection with sterile tools inside a laminar flow cabinet, to prevent contamination.
- DNA extraction using a commercial DNA kit.
- Quantitative Real-Time PCR (qRT-PCR) targeting the 16S rRNA gene.
- Sequencing preparation underway for bacterial identification.



RESULTS

Anthropological and Oral Pathological Findings:

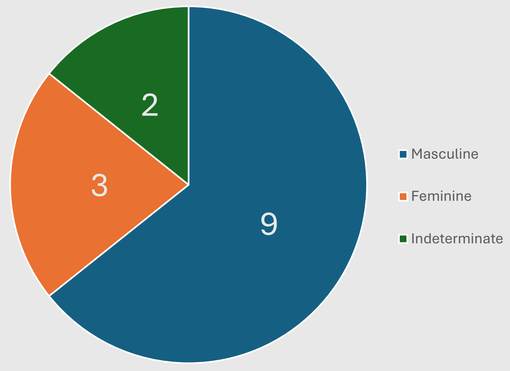


Figure 4: Sex Distribution of the analysed individuals

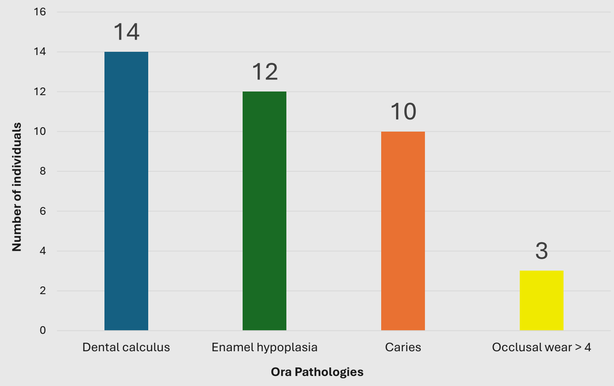


Figure 5: Combined Prevalence of Oral Pathologies in 14 individuals

Microbiological Findings:

- Microbial DNA successfully detected in all samples via qRT-PCR.
- Amplification curves confirm the preservation and integrity of microbial DNA.

DISCUSSION

- The presence of multiple oral pathologies suggests dietary and health-related stress within the community but also poor oral hygiene.
- Occlusal wear indicate the consumption of abrasive food or the use of teeth for non-masticatory purposes.
- Despite the absence of sequencing data, the preliminary results validate the methodology employed.

CONCLUSIONS

- This study demonstrates the value of an interdisciplinary approach that combines bioanthropology and molecular biology to investigate the health and lifestyle of past populations.
- Dental calculus is a reliable and informative substrate for the recovery of ancient microbial DNA.
- The anthropological and pathological data enhance our understanding of the individuals' biological profiles and oral health, supporting integrated bioarchaeological approaches and shedding light on past lifestyles in Portugal.

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