

# Bacteriome analysis in ocular MALT lymphoma by 16S rRNA gene sequencing: A case report





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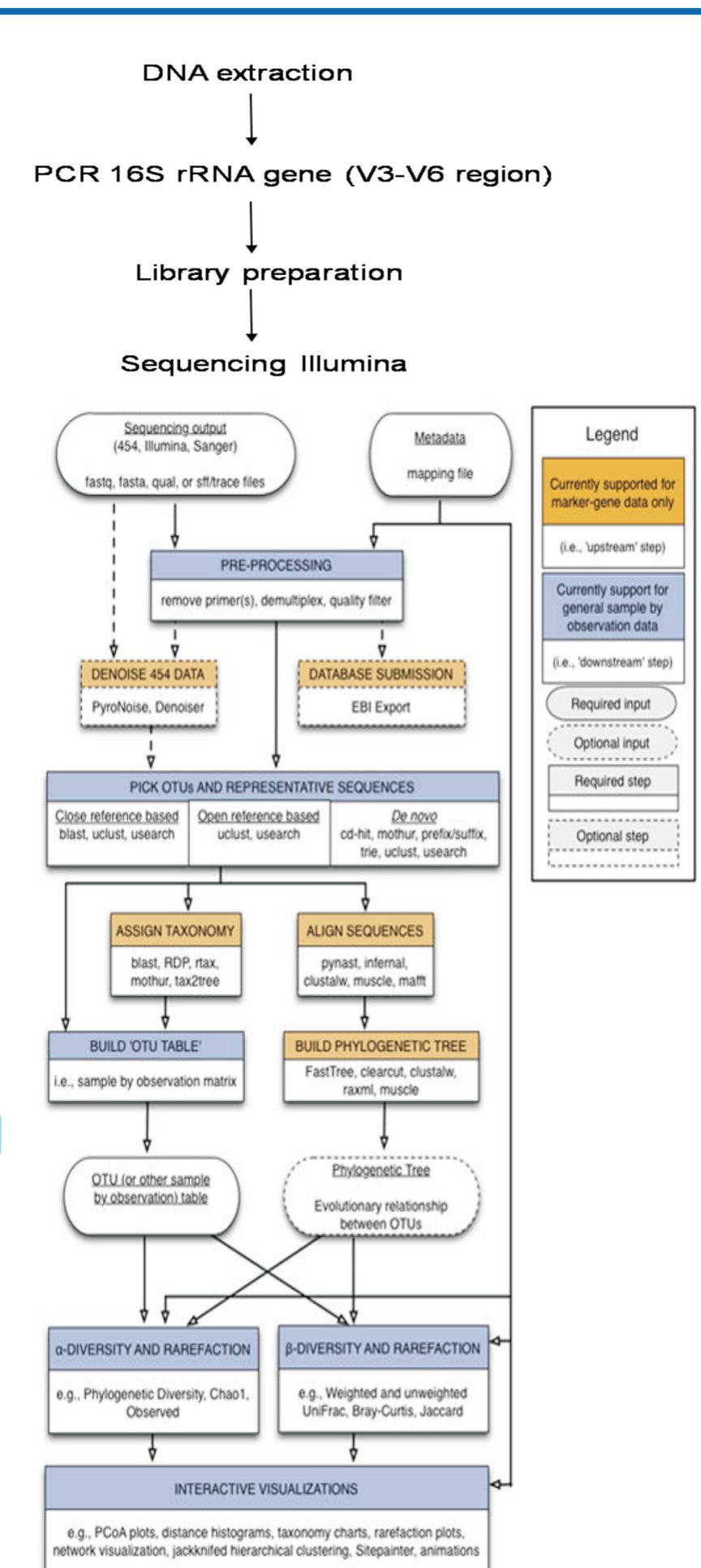
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# PURPOSE

Primary ophthalmic lymphoma represents 5-15% of all extranodal and 1- 2% of all non-Hodgkin's lymphomas (NHL). Mucosaassociated lymphoid tissue (MALT) is a common histologic type of primary ophthalmic non-Hodgkin`s lymphomas, accounting for 38-100% of ocular adnexal NHL. MALT lymphoma arises in lymphoid tissue at extranodal sites as a result of chronic inflammation or autoimmune disorders. However, etiologic agents in ocular MALT remain undefined. Presence of *Chlamydia* psittaci in ocular MALT lymphoma has been reported with variable prevalence in different populations, generating conflicting assumptions about *C. psittaci* as a causative agent of ocular MALT lymphoma. Tumor regression after doxycycline therapy supports the hypothesis of bacterial infection contributing to the development of ocular MALT. Additionally, regression of lymphoma lesions after doxycycline therapy in C. psittaci DNA-negative patients suggests other doxycyclinesensitive bacteria may play a role in the pathogenesis of ocular MALT. In this study, we performed bacteriome analysis in both, ocular MALT lymphoma and control eye, of a 57 years-old patient using high-throughput bacterial 16S rRNA gene sequencing for analysis of conjunctiva swabs before and after 3 weeks of doxycycline treatment.

# METHODS

Total genomic DNA was extracted from swab conjunctiva sample collected from both eyes, affected and control, before and after treatment with doxycycline antibiotic. Genetic material was amplified and sequenced by **Illumina Hiseq 2500 platform**. Operational taxonomic units (OTUs) were assigned to the reads sequence using 16S gene database (Greengenes database, August 2013 version) in QIIME (Caporaso et al., 2010). OTUs with frequency lower than 0.005% was discarded and those remaining were summarized at the phylum, class, order, family, genus and species.



# RESULTS

the antibiotic treatment.

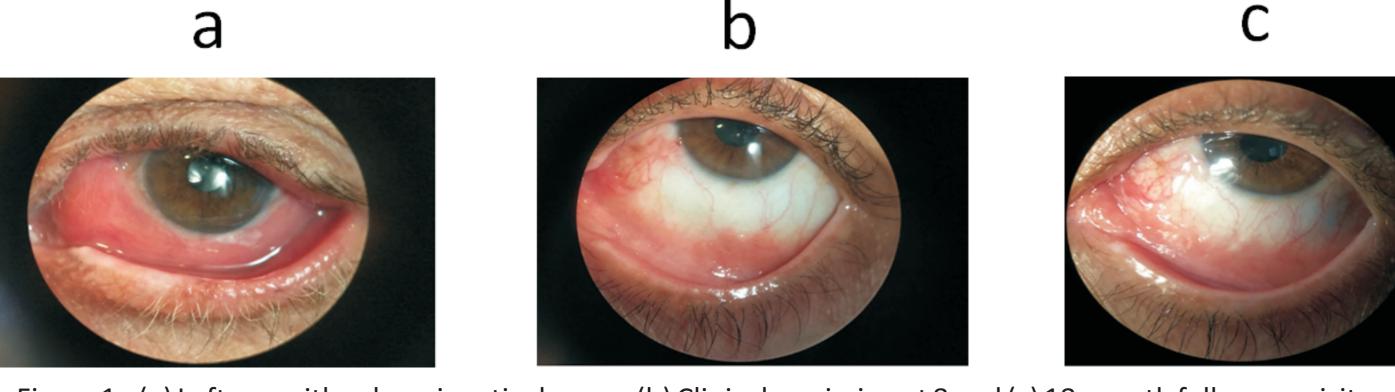


Figura 1- (a) Left eye with subconjunctival mass. (b) Clinical remission at 8 and (c) 18-month follow-up visit.

An Incisional biopsy was performed after conjunctival swab in both eyes. Immunohistochemistry confirmed diagnosis of ocular MALT lymphoma. The sample collected from affected eye before the treatment showed high relative abundance of the Neisseriasea family when compared to the control eye sample collected at the same time point. Interestingly, there was a reduction in abundance of this family after the antibiotic treatment.

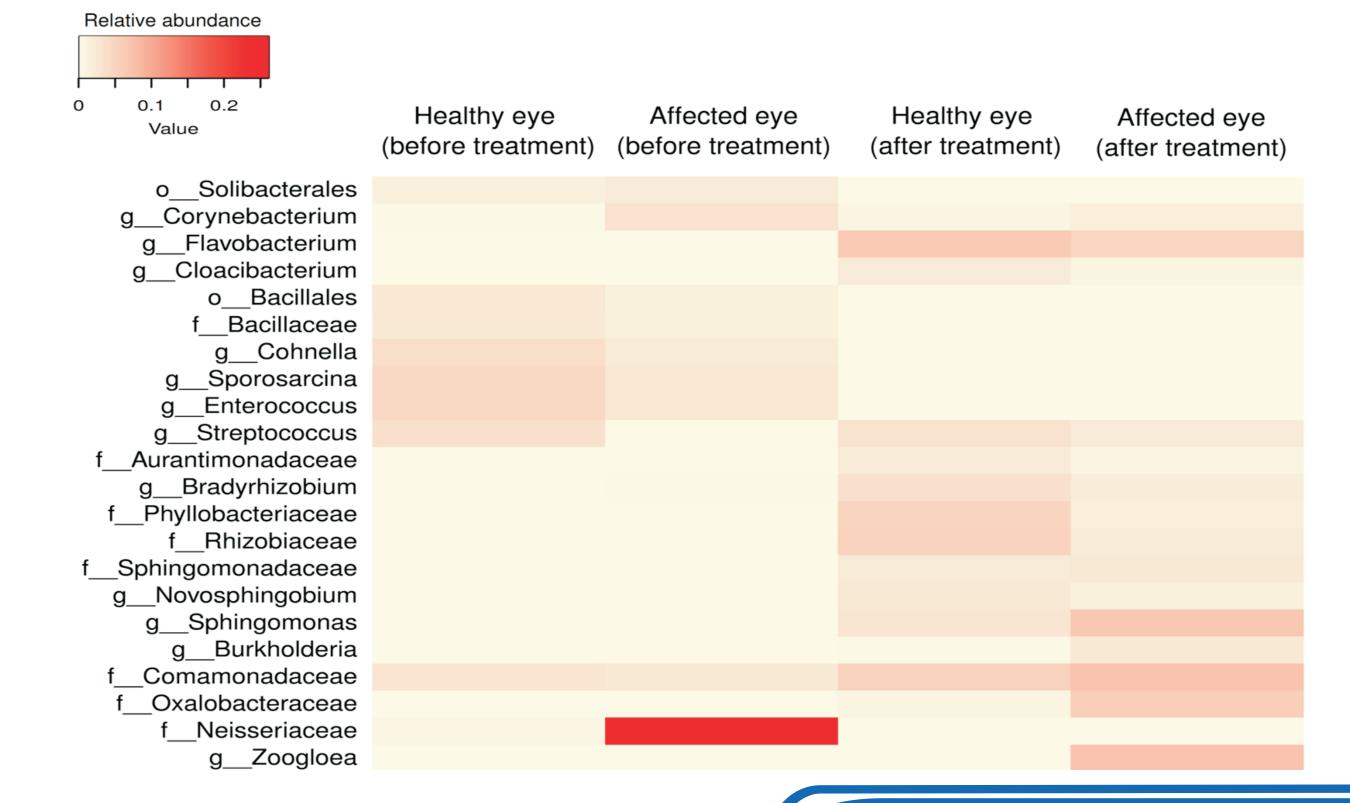


Figura 3- Heatmap generated by unsupervised hierarchical clustering analysis of ocular microbiome before and after

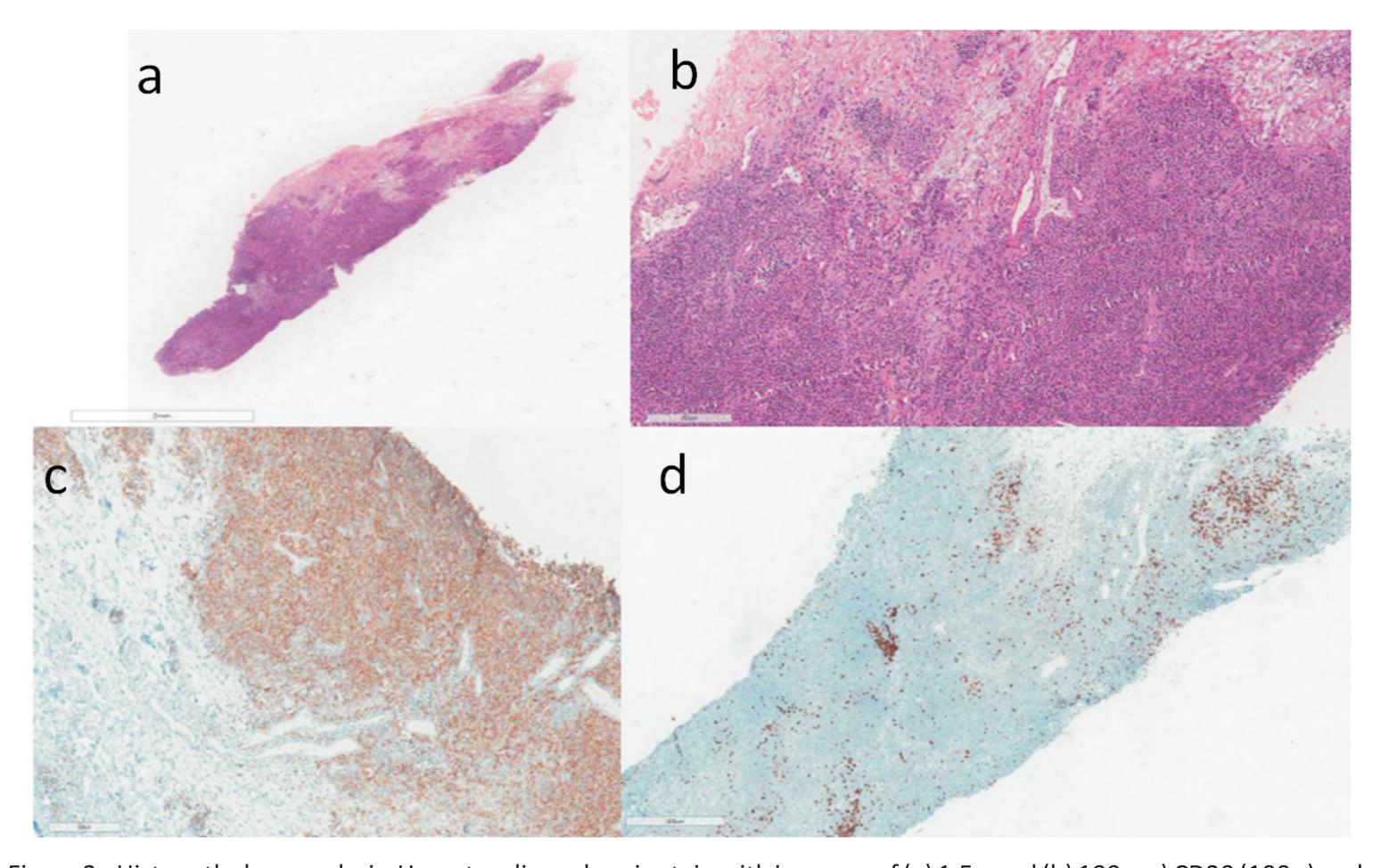


Figura 2- Histopathology analysis. Hematoxylin and eosin stain with increase of (a)  $1.5 \, x$  and (b)  $100 \, x$ . c) CD20 ( $100 \, x$ ) and (d) KI67 ( $100 \, x$ ) immunohistochemical staining.

## CONCLUSION

We describe, for the first time, the predominance of Neisseriaceae bacteria in ocular MALT lymphoma, clinical evolution and microbiological assessment overtime.

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Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA





