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Genome-sequencing of ocular fluid in a patient with syphilitic uveitis: Strain identification and antimicrobial resistance detection

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ABSTRACT

Purpose: To describe an unusual case of bilateral, hypertensive uveitis in which a syphilis genome with antimicrobial resistance was identified in the aqueous humor.
Methods: A retrospective review of patient records was performed for clinical data. An aqueous humor sample was collected, and syphilis genomes were analyzed for phylogenetic traits and antimicrobial resistance.
Results: A 64-year-old male was referred to our uveitis service for an evaluation of bilateral hypertensive anterior uveitis. On examination, visual acuities were 20/80 in the right eye (OD) and 20/100 in the left eye (OS), and intraocular pressure was 57 mmHg OD and 56 mmHg OS. Ophthalmic examination revealed severe conjunctival injection, severe anterior chamber inflammation, and vitreous opacities bilaterally. A uveitis laboratory work-up revealed a reactive syphilis IgG and an elevated RPR. Because of ongoing concern of a viral process, PCR testing was performed for HSV, VZV, and CMV, which were negative. Subsequent genomic sequencing of the residual ocular fluid demonstrated *Treponema pallidum* species, which showed the Nichols lineage with macrolide resistance. The patient responded favorably to penicillin therapy with resolution of syphilitic uveitis and improvement in visual acuity.
Conclusions and importance: This case report highlights the utility of ocular fluid's genome sequencing in diagnosing ocular syphilis and detecting antimicrobial resistance. Given the resurgence of syphilis in the U.S. and globally and the shortage of penicillin worldwide, genomic sequencing of ocular fluid may also assist in antimicrobial resistance detection for syphilis, along with pathogen identification, in concert with serologic assessment.

1. Introduction

Syphilis is a sexually transmitted infection caused by the spirochete *Treponema pallidum (T. pallidum)*, which may involve multiple organs, including the eyes and central nervous system. The incidence of syphilis is expanding globally, as well as in the United States, and it is estimated that about 12 million new cases occur globally per year with most cases in developing countries.[1] In the United States, the rates of syphilis dropped dramatically between 1990 and 2000; however, the prevalence of syphilis has substantially increased since 2020, with increased rates in men having sex with men (MSM).[2,3] In 2023, 209,249 cases were reported in the United States, which represents an increase from the 133,965 cases reported in 2020 according to the Center for Disease Control and Prevention (CDC).[4]

Ocular syphilis can present with a broad spectrum of ocular manifestations affecting any anatomic location, but posterior uveitis and

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panuveitis are the most common.[5] The pathogenesis of ocular syphilis is underexplored; however, previous studies have hypothesized the hematogenous dissemination of the bacteria to the ocular structure, leading to the ocular manifestations.[6] Different strains of syphilis have been isolated in the literature,[7,8] and recently, a cluster of five cases of ocular syphilis in women with a common male sex partner was identified in Michigan, suggesting the possibility of a *T. pallidum* strain with ocular tropism.[9]

Since its introduction in early 2000s, next-generation sequencing has expanded its utilization in ophthalmology with a number of studies demonstrating its capacity in the detection of common and rare etiologies of infectious uveitis.[10–13] A prior study by Doan et al. was able to detect rubella virus, toxoplasmosis and herpes simplex virus type 1, as causes of uveitis via next-generation sequencing.[12] Mutations in genes, which confer antimicrobial resistance in treatment of cytomegalovirus uveitis and ocular syphilis, have also been detected in prior studies.[11,13] Herein, we report the utility of genomic sequencing in the diagnosis of a patient with syphilitic uveitis and present the sequencing results from the patient's ocular fluid. This case further illustrates how genomic tools can aid in both confirming diagnosis and identifying potential resistance mutations, which may guide treatment decisions in the setting of increasing antimicrobial resistance[14] and the potential for therapeutic shortages, including penicillin.[15]

2. Materials and methods

2.1. Clinical data and sequencing

The patient was evaluated at the Stanley M. Truhlsen Eye Institute, University of Nebraska Medical Center. A review of patient records was performed for data including past medical history, past surgical history, ophthalmic examination findings, and multimodal imaging findings. Ocular fluid sample was collected via anterior chamber (AC) paracentesis and enrolled in a protocol (University of Nebraska Medical Center IRB# 0670-21-EP) for ocular fluid analysis.

The ocular fluid sample was subjected to nucleic acid extraction using the MagMax Viral/Pathogen Ultra Nucleic Acid Isolation Kit (ThermoFisher Scientific Inc, Waltman, Massachusetts; Cat. A42356) following the manufacturer's protocols. The sample was initially prepared for sequencing using a hybrid-capture enrichment approach targeting viral pathogens, as this was the initial suspected etiologic agent given the presentation of an acute, hypertensive uveitis. Samples were prepared using Illumina's DNA Prep with Enrichment kit (Illumina Inc, San Diego, California; Cat. 20025524) following manufacturer's protocols with the Twist Biosciences Comprehensive Viral hybridization probe panel (Twist Biosciences, South San Francisco, California; Cat. 103548). The sample was first sequenced on the Illumina iSeq 100 platform using the i1 cartridge with 2x150 paired-end sequencing (e.g., short read sequencing).

After detection of *T. pallidum* in our initial sequencing, we enriched *T. pallidum* nucleic acids from the sample using enrichment methods. Extracted nucleic acid was subjected to selective whole genome amplification (SWGA) as previously described.[16] Following SWGA, samples were cleaned using KAPA beads (Roche Holding AG, Basel, Switzerland; Cat. 07983280001) with a 0.5X ratio, quantified with a Qubit 4 (ThermoFisher Scientific Inc, Waltman, Massachusetts). Amplified nucleic acid was subjected to both long and short read sequencing. For short read sequencing, the SWGA nucleic acid was subjected to the Illumina DNA Prep without enrichment (Illumina Inc, San Diego, California; Cat. 20025520) and sequenced Illumina iSeq 100 platform using the i1 cartridge with 2x150 paired-end sequencing. For long read sequencing, we used the Oxford Nanopore Technology (ONT) MinION platform. Libraries were prepared with the Ligation Sequencing V14 kit (Oxford Nanopore Technologies pIc, Oxford, United Kingdom; Cat. SQK-LSK114) and sequenced using a R10.4.1 flow cell (Oxford Nanopore Techonologies pIc, Oxford, United Kingdom; Cat. FLO-MIN114).

2.2. Bioinformatic analyses and phylogenetics

Illumina paired-end short reads and ONT long reads were filtered for *Treponema* genus-specific sequencing reads using the standard Kraken 2 v2.0.8 database (March 2019)[17] followed by trimming with Trimmomatic[18] v0.39. Illumina reads were required to have both a forward and reverse read pair classified to *Treponema* in order to be considered for assembly to reduce the presence of potentially contaminating bacterial reads, which can lead to conflicting results specifically in highly conserved regions like the 23S.[7,8] ONT reads classified to *Treponema* with Kraken2 at a confidence threshold of 0.2 were retained for downstream assembly for the same contamination reason.

Illumina short reads were subsequently used to assess macrolide resistant Single Nucleotide Polymorphisms using Antimicrobial Resistance Identification By Assembly (ARIBA),[19] which performs local assembly and variant calling using a custom reference database containing the 23S sequences from the Nichols (NR_076156.1) and SS14 (NR_076531.1) reference strains, as previously reported.[8] These data were used to infer the presence/absence of both A2058G and A2059G 23S variants that confer macrolide resistance.

Subsequently, a custom reference-based assembly was used to compile the genome from this study using the *Treponema*-only classified long Nanopore reads (https://github.com/rchapman2000/ref-based-assembly-pipeline) and the SS14 v2 reference (NC_021508.1). Briefly, reads were aligned to the reference using Minimap2 (v2.24),[20] polished using Medaka v1.7.2 (https://github.com/nanoporetech/medaka), variant calling and consensus generation were performed using samtools v1.16.1,[21] longshot v0.4.5,[22] and bcftools v1.16,[21] requiring a minimum of five supporting reads to call a variant (minCov = 5x). The resulting near-complete genome (98.32 % reference coverage) had an average read depth of 494.31x. ARIBA only supports paired-end Illumina reads, however, manual inspection of long ONT reads in Geneious v2025.1 confirmed the presence of the A2058G mutation in the assembled genome.

Publicly available genome assemblies were obtained from the National Center for Biotechnology Information (NCBI) to perform phylogenetic characterization of the sample from this study. Prior to aligning all sequences, masking of 14 highly repetitive or recombinogenic genes was performed as described previously,[8] and then aligned using MAFFT v7.526.[23] Multiple sequence alignments were screened for recombination evidence using Gubbins v 3.4,[24] and the maximum likelihood phylogeny was calculated on SNP-only alignments using IQ-Tree (v3.0.1).[25] This approach allows for built-in model testing to determine a TVMe + ASC + R2 (allows four different transversion rates and one shared transition rate plus equal base frequencies) substitution model, with a FreeRate model of heterogeneity, and performing 1000 UltraFast Bootstraps as previously described.[8]

Data processing was performed in R v 4.5.0 (R Core Team, 2014),[26] and phylogeny was plotted using ggtree.[27] Figures were produced using ggplot2[28] and finalized in Inkscape v 1.4.2.

3. Results

A 64-year-old male was referred to our uveitis service for evaluation of bilateral hypertensive anterior uveitis. Five days before presentation to our clinic, he was evaluated by an outside ophthalmologist for 2 weeks of blurry vision in both eyes (OU). At that time, best-corrected visual acuity (BCVA) was 20/50 in the right eye (OD) and 20/70 in the left eye (OS), and intraocular pressure (IOP) was 52 mmHg OD and 50 mmHg OS. Slit-lamp examination revealed 3–4+ AC cells with scattered fine keratic precipitates (KPs) OU. The patient was diagnosed with bilateral hypertensive anterior uveitis, and he was started on topical prednisolone acetate, brimonidine/timolol, dorzolamide, bimatoprost, and oral acetazolamide. At that time, he was taking 40 mg prednisone for polyarthralgia and facial swelling following the monkeypox vaccine (JYNNEOS™, Bavarian Nordic A/S). Subsequently, he

was urgently referred to our uveitis service for further evaluation.

The patient's past medical history included atrial fibrillation, essential hypertension, hyperlipidemia, nonspecific polyarthralgia, right corneal abrasion, and treated gonorrhea. His past surgical history was pertinent for appendectomy, coronary artery bypass grafting, tonsillectomy, and adenoidectomy. Family history was unremarkable for any ocular diseases. The patient was in a same-sex relationship and endorsed participating in high-risk sexual activities outside of his relationship. On examination, visual acuities were 20/80 OD and 20/100 OS, and IOP was 57 mmHg OD and 56 mmHg OS. Pupil, confrontational visual fields, extraocular movement, and relative afferent pupillary defect testing were unremarkable. Slit lamp examination revealed 2–3+ conjunctival injection, 3+ AC cells, and 2+ nuclear sclerosis OU. Fundus examination was limited due to vitreous opacities OU (Fig. 1A and B). Optical coherence tomography (OCT) revealed significant vitreous opacities OU, but no evidence of intraretinal or subretinal fluid was noted OU (Fig. 1C and D). The patient was diagnosed with anterior and intermediate uveitis OU. After an extensive discussion with the patient, a comprehensive uveitis work-up was performed including angiotensin-converting enzyme (ACE), lysozyme, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), HLA-B27, QuantiFERON-TB Gold, antineutrophil cytoplasmic antibodies (ANCA), human immunodeficiency virus (HIV), rapid plasma reagin (RPR) and syphilis immunoglobulin G (IgG). Oral valacyclovir was also added to the therapy regimen due to concern for herpetic uveitis.

Two days after his initial presentation to our service, the patient reported minimal improvement in his symptoms. BCVA was 20/200 OD and 20/30 OS, and IOP was 53, and 47 mmHg OD, and OS, respectively. Because of the concern for herpetic uveitis due to elevated IOP, an AC paracentesis was performed for PCR testing for VZV, HSV, and CMV, and the residual ocular fluid was used for genomic sequencing. Oral prednisone was discontinued pending laboratory testing for infectious etiology. Laboratory testing revealed a positive syphilis IgG with a reflex rapid plasma reagin (RPR) quantitative of >1:256, and the patient was admitted to the hospital for further management of ocular syphilis. Given the patient's clinical presentation, it remained unclear whether syphilis was responsible for the uveitis versus a viral process.

The patient's uveitis work-up including QuantiFERON-TB Gold, ACE, lysozyme, CRP, ANCA, and HIV were within normal limits. The ESR was elevated at 67. The AC paracentesis was negative for VZV, HSV, and CMV. Urine testing was negative for chlamydia and gonorrhea. During hospitalization, brain MRI and chest X-ray were performed, and they were unremarkable. The patient was unable to tolerate IV penicillin in the first two days of the hospitalization due to a burning sensation from the peripherally inserted IV catheter and was switched to ceftriaxone. The patient was then switched back to IV penicillin, which he tolerated well via a midline catheter. The patient was also treated concurrently with topical prednisolone acetate four times daily OU.

Upon completing the 14-day penicillin course, he reported significant improvement in his visual acuity and symptoms. The patient's BCVA was 20/25 OU, and IOP was 18 and 17 mmHg, OD and OS, respectively. Slit lamp examination revealed rare AC pigmented cells OD and no cell OS. Fundus examination showed mild vitreous opacities, optic nerve edema, and several white retinal lesions temporal to the macula OU (Fig. 2A and B). Late-phase fluorescein angiography revealed staining of the corresponding retinal lesions OD and bilateral hyperfluorescence of the optic disc OU (Fig. 2C and D). OCT was unremarkable OU (Fig. 2E and F). After an extensive discussion with the patient about the ongoing disease findings and concerns that the patient had late latent syphilis, the patient received another dose of 2.4 million units of intramuscular benzathine penicillin G. At the same time, topical prednisolone acetate was continued, and he was also advised to continue medications for ocular hypertension. The patient continued to be followed closely with gradual improvement in his symptoms during this time. On the 7-month follow-up visit after the initial visit at our clinic, BCVA improved to 20/20 OU with IOPs of 25 and 22 mmHg, OD and OS, respectively. Slit lamp examination showed no AC cell or flare OU, and fundus examination demonstrated resolution of retinal lesions OD and optic nerve edema OU. Repeat RPR at this visit was 1:16. In the most recent visit, 2 years after the initial visit, vision remained stable OU.

Sequencing of ocular fluid from the patient and phylogenetic analysis performed with the sample from this study plus 79 other publicly available genomes demonstrated *T. pallidum* consistent with the Nichols lineage (Fig. 3). Mutation analysis of the sample also revealed the presence of an A2058G point mutation in the 23S ribosomal gene, conferring resistance to macrolide antibiotics (Fig. 3).

4. Discussion

Given the potential clinical implications of strain variability, the role

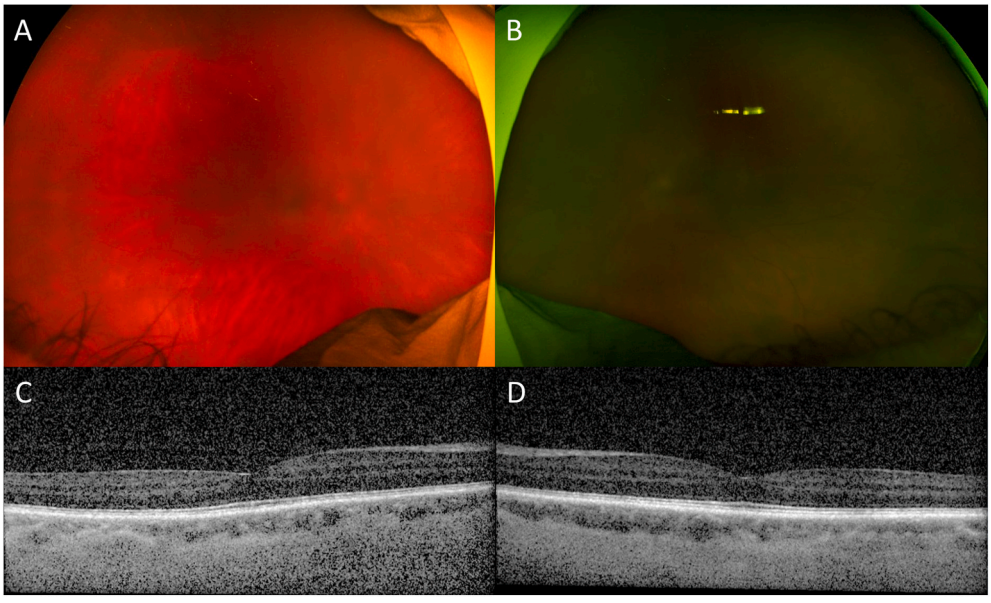

Fig. 1. Ultrawide-field fundus photo showed significant media and vitreous opacities OU with limited view of fundus **(A, B)**. Optical coherence tomography revealed significant vitreous opacities OU, but no other abnormalities **(C, D)**.

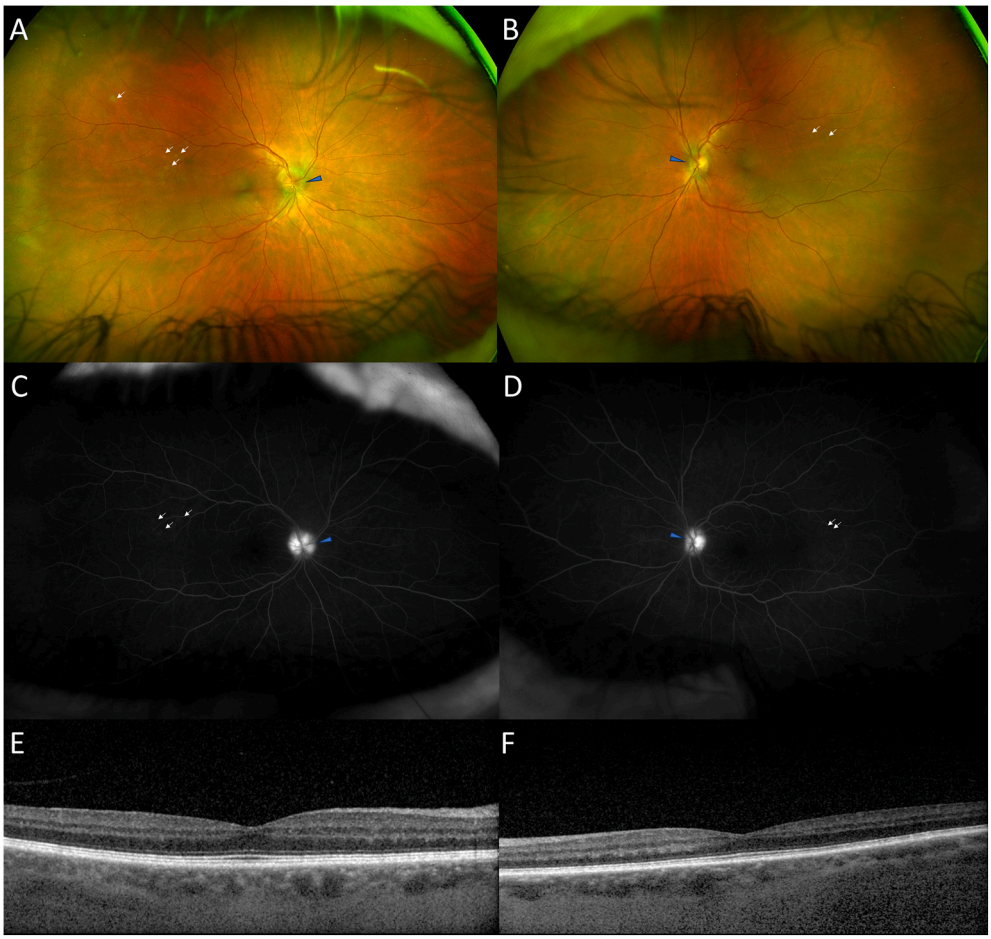

Fig. 2. Ultrawide-field fundus photo demonstrated mild vitreous opacities, optic nerve edema (blue triangle), and several white retinal lesions (white arrows) temporal to the macula OU **(A, B)**. Late-phase fluorescein angiography revealed hyperfluorescent staining of the corresponding retinal lesions (white arrows) OD and bilateral hyperfluorescence (blue triangles) of the optic disc OU **(C, D)**. The OCT scans were unremarkable **(E, F)**.

of genomic sequencing in syphilis diagnosis may be relevant to cases of atypical presentations, potential antimicrobial resistance, or treatment failure. Although syphilis has been responsive to penicillin and resistance to penicillin has not been reported in the literature previously, the potential for macrolide resistance in the setting of penicillin shortages and the high prevalence of penicillin intolerance are important considerations in management. While azithromycin has not been used for ocular syphilis in the literature, several previous studies showed comparable efficacy of azithromycin in treating all stages of syphilis in comparison to penicillin.[29] However, the recently increased azithromycin-resistant strains have proven to be problematic for the potential use of azithromycin in treating syphilis.[8,30] Reportedly, *T. pallidum* has primarily developed antibiotic resistance through point mutations, rather than gene transfer mechanisms such as transposases, due to its compact genome.[31] The most commonly described mutations associated with drug resistance in clinical settings are A2058G and A2059G within the 23S rRNA gene, which confer macrolide resistance.[31] Studies have also suggested changes in the genes encoding penicillin-binding proteins may portend future penicillin resistance.[8] Recently reported cases of ocular syphilis related to a sexual partner shared amongst multiple individuals who developed ocular syphilis also suggest that specific strains may be associated with an increased likelihood of ocular syphilis.[9] Further genome sequencing studies may provide insight into these phenomena.

Globally, therapies for syphilis are heavily reliant on injectable benzathine penicillin G, the first-line therapy for all stages of the disease. In recent years, however, supply chain fragility and low market profitability have contributed to recurring shortages of penicillin,[15] with only one manufacturer currently supplying it in the United States.[32] This shortage is particularly impactful for regions with high syphilis prevalence, as benzathine penicillin is the most effective treatment for ocular syphilis, as well as other forms of syphilis including congenital syphilis—a condition associated with devastating outcomes, including fetal demise in approximately one-third of affected pregnancies.[33]

Ocular syphilis can present with a wide range of ocular presentations, most commonly posterior uveitis and panuveitis. Although the diagnosis of ocular syphilis can be challenging due to its variable clinical presentations mimicking other diseases, characteristic multimodal imaging findings and laboratory testing can aid clinicians in establishing a diagnosis.[34,35] Our patient was diagnosed with bilateral hypertensive anterior uveitis that was initially unresponsive to corticosteroids and topical anti-hypertensive medications. Our initial concern for a viral etiology prompted an AC paracentesis for PCR testing, which led to the sequencing of *T. pallidum* within the ocular fluid. The patient responded well to antimicrobial therapy with resolution of his clinical symptoms and improvement of visual acuity at long-term follow-up. The patient's atypical presentation highlights the ability of syphilis to masquerade other conditions and emphasizes the importance of close evaluation in patients with risk factors for syphilis such as MSM, history of HIV, high-risk sexual behaviors, and recreational drug use. In addition, the genomic sequencing of the ocular fluid sample provides further insight into the importance of the technology in establishing a diagnosis in patients with challenging disease presentations.

Our current understanding of *T. pallidum* pathobiology comes from

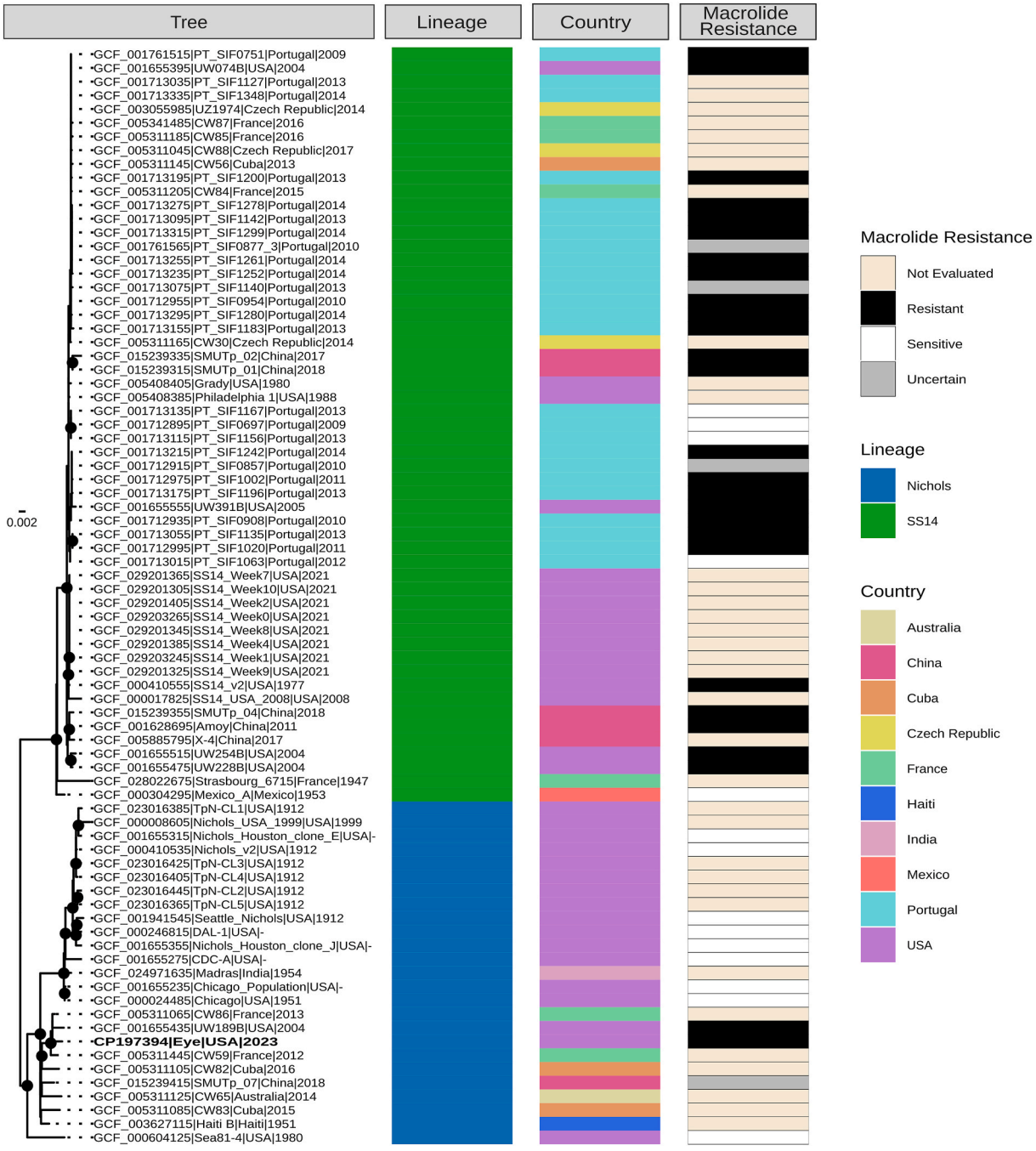

Fig. 3. Maximum likelihood phylogeny of 80 *T. pallidum* subspecies *pallidum* genomes, showing major lineage, country of origin, and macrolide resistance. Tree includes sample recently sequenced from ocular fluid highlighted in bold. Ultra-fast bootstrap value $\geq$ 95 % are labeled with black node points. Branches are scaled by mean nucleotide substitutions/site indicated to the left of the tree. Macrolide resistant SNPs were inferred using ARIBA, which performs localized assembly and mapping in comparison with a custom reference database containing 23S sequences from Nichols (NR_076156.1) and SS14 (NR_076531.1) reference strains.

related species or from *T. pallidum* cultured in the in vivo rabbit testicular model because of low concentration of bacterial pathogen in clinical specimens.[36] While the precise pathogenesis of ocular disease in syphilis is incompletely understood, studies postulate the hematogenous dissemination of the bacteria in ocular tissues with subsequent invasion of ocular structures.[6] The bacteria have also been suggested to cross retina-blood-barriers through its migration through the endothelium,[37–39] with a resultant immunologic response to the spirochetal invasion with the predilection for the posterior segment including the choroid, retina, and optic nerve led to the clinical manifestations of ocular syphilis. Identification of a high concentration of syphilitic genomes in our case, as well as in other studies, supports the presence of bacteria in ocular fluid as a driver of disease with severe associated inflammation.[37,38]

Our atypical case of syphilitic uveitis and sequencing results that demonstrated the Nichols lineage of syphilis with macrolide resistance illustrates the clinical utility of sequencing to understand syphilitic uveitis further. Moreover, the identification of antimicrobial resistance has implications for management, especially in the setting of penicillin shortage or patient intolerance.

CRediT authorship contribution statement

Nam V. Nguyen: Writing – review & editing, Writing – original

draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Jessica Wiley:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Paul Garcia:** Writing – review & editing, Writing – original draft. **Tolulope Fashina:** Writing – review & editing, Investigation. **Hythem Abouodah:** Writing – review & editing, Investigation. **Timothy Kaftan:** Writing – review & editing, Investigation. **Christopher D. Conrady:** Writing – review & editing, Investigation. **Shaun T. Cross:** Writing – review & editing, Methodology, Investigation. **Michael R. Wiley:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Steven Yeh:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Statement of informed consent

The written informed consent for patient information was obtained.

Ethical approval

This case report was conducted in accordance with the Declaration of Helsinki. The collection and evaluation of all protected patient health information was performed in a Health Insurance Portability and Accountability Act (HIPAA)-compliant manner.

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajoc.2025.102486.

Data availability

Raw sequencing reads were deposited at the NCBI under BioProject PRJNA1300384 and BioSample accession SAMN50360482 with Sequencing Read Archive (SRA) accessions SRR34832874 for Illumina reads and SRR34832873 for ONT MinION reads. The genome from this study (assembled using only ONT MinION reads) was also deposited to NCBI under accession number CP197394. All publicly available genomes used in this study are listed in Supplementary Data Table 1.

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