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RESEARCH

Molecular investigation of unexpected *Helicobacter pylori* bacteremia

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Abstract
This study describes the isolation of *Helicobacter pylori* from a blood sample of an 88-year-old patient with no history or clinical signs suggestive of this infection. We summarize the microbiological characterization of the case, and the subsequent investigation using advanced next-generation sequencing methods, including a target enrichment technique adapted for paraffin-embedded gastric biopsy analysis.

Keywords *Helicobacter pylori* · Bacteremia · Next-generation sequencing

Introduction

Helicobacter pylori infection affects approximately half of the global population and is well recognized for its role in gastroduodenal diseases, particularly gastric ulcers. Despite its prevalence, *H. pylori* infection typically remains confined to the stomach and is rarely associated with bacteremia, with only a few cases reported in the literature to date.

In 2006, Huang et al. [1] identified *H. pylori* DNA in the peripheral blood of three patients with duodenal ulcers using polymerase chain reaction (PCR). Gastric biopsy specimens from these three patients confirmed the infection, as *H. pylori* was successfully cultured and identified through *16 S rDNA* gene sequencing. This study suggested that *H. pylori* bacteremia can occur in immunocompetent individuals [1]. In another case, Han et al. [2] described a 65-year-old woman with gastric bleeding and breast cancer who underwent antineoplastic chemotherapy. Although *H. pylori* was not cultured from her blood, the bacterium was identified through *16 S rDNA* sequencing. Notably, her fever resolved without specific anti-*H. pylori* treatment [2].

More recently, in 2023, a metagenomic next-generation sequencing (NGS) approach identified *H. pylori* in the blood of a woman with no relevant medical history. Attempts to subculture the bacterium on solid media were unsuccessful [3]. This study highlighted the utility of modern NGS techniques in detecting *H. pylori* bacteremia.

We present herein a case of *H. pylori* bacteremia diagnosed at Lyon University Hospital in an 88-year-old patient with disseminated vascular purpura and a biological inflammatory syndrome of unknown origin. Blood culture was positive for *H. pylori*, and the infection was confirmed via gastric biopsy histology. Additionally, we detail how modern NGS methods, conducted at the French National Reference Center for Campylobacters and Helicobacters (NRCCH), were instrumental in fully characterizing this case.

Materials and methods

Bacterial culture

The *H. pylori* strain that was initially isolated from the patient's blood at Lyon University Hospital and sent to the NRCCH was cultured following standard laboratory procedures [4]. The strain was grown on in-house blood agar

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supplemented with antibiotics and incubated at 36 °C for 10 days in a microaerobic environment using a specialized workstation (Baker Ruskinn Concept, Bridget+; Baker Ruskinn, Bridgend, UK).

The plates were examined daily starting on day 2, and colonies were assessed using oxidase, catalase, and urease tests as well as morphological observation. Antimicrobial susceptibility testing was conducted on Mueller-Hinton agar containing 10% sheep blood and freshly prepared globular extract. Etest® strips (bioMérieux, Marcy-l'Étoile, France) were used to determine minimum inhibitory concentrations (MICs) for clarithromycin, levofloxacin, rifampicin, and tetracycline. MIC values were interpreted based on the guidelines of the French Microbiology Society Antibiogram Committee (CA-SFM/EUCAST: Société Française de Microbiologie, Ed. 2024: p. 1–177).

MIC readings were performed independently by two readers, and quality control was ensured using the reference *H. pylori* strain CCUG 17,874.

Whole-genome sequencing of *H. pylori* strain isolated from blood

Whole-genome sequencing of the *H. pylori* strain isolated from the patient's blood was performed using the GenoBioMICS sequencing platform (CHU Henri Mondor, Créteil, France). Total DNA was extracted using a combination of mechanical (bead-beating), enzymatic, and chemical methods on a Chemagic Prime instrument (Revvity, Waltham, MA, USA). Libraries were prepared with aDNA Prep Kit (Illumina, Inc., San Diego, CA, USA) on a DreamPrep NGS automaton (Tecan Group Ltd., Männedorf, Switzerland) and sequenced using a NovaSeq 6000 platform (Illumina, Inc.).

Bioinformatic analysis was performed following the same previously described pipeline, with the exception of the genome assembly step, which was performed using SKESA v2.5.1 (NCBI, Bethesda, MD, USA) [5]. A supplementary annotation step was included to extract genomic sequences of the *cagA* gene, utilizing Prokka v1.14.5 [6].

Table 1 Mutations associated with phenotypic resistance to the antibiotics studied

Gene	Mutations	Associated resistance
16 S rDNA	$AGA_{926-928}TTC$, $AGA_{926-928}ATC$, $AGA_{926-928}TTA$, $AGA_{926-928}TGC$, $AGA_{926-928}AGC$, $AGA_{926-928}ATA$	Tetracycline
23 S rDNA	A2142C, A2142G, A2143C, A2143G	Clarithromycin
QRDR *(gyrA)*	N87K, N87I, N87Y, A88P A88V, D91G, D91N, D91Y	Levofloxacin
rpoB	L525I, L525P, Q527K, Q527R, D530E, D530V, D530N, D530G, H540Y, H540N, S545L, L547F, I586N, I586L	Rifamycin

Probe design for target enrichment

An RNA probe library consisting of 13,245 120-mer probes, totaling 108 kbp, was designed by Agilent (Santa Clara, CA, USA). This probe library was specifically developed to detect genomic mutations associated with antimicrobial resistance, including mutations in *23 S rDNA* (clarithromycin resistance), *gyrA* (levofloxacin resistance), *rpoB* (rifampicin resistance), and *16 S rDNA* (tetracycline resistance) (Table 1). Additionally, the probe design targeted regions commonly used in the *H. pylori* multilocus sequence typing (MLST) scheme, as described by Falush et al. [7, 8].

Automated capture-based library Preparation

Most steps of the SureSelectXT workflow were automated using Agilent's MagnisDx library preparation system, from enzymatic fragmentation to the capture of hybridized libraries. DNA extracted from formalin-fixed, paraffin-embedded (FFPE) biopsy samples was normalized to 100 ng in a 14-µL input volume. Following Agilent's recommendations, enzymatic fragmentation was performed for 20 min, optimized for high-quality DNA and a read length of 2 × 150 base pairs. The experimental workflow included 18 pre-capture PCR cycles and 24 post-capture PCR cycles in accordance with the manufacturer's guidelines.

NGS short-read sequencing

The fragment size distribution and molarity of the final libraries were assessed using Agilent's TapeStation 4150 system with the High Sensitivity D1000 assay. A 2-µL aliquot of the library was diluted 10-fold and mixed with 2 µL of High Sensitivity D1000 Sample Buffer, then normalized to an equimolar concentration of 0.125 nM. A 20-µL aliquot of the final mix was prepared for multiplex sequencing, which was performed on the Illumina iSeq 100 sequencer at the NRCCH.

Bioinformatics workflow

Raw reads were first mapped to the *Homo sapiens* GRCh38 reference genome using Bowtie2 v0.7.17 to exclude host-derived sequences. Unmapped reads were trimmed and cleaned with fastp v0.23.4 [9], and a deduplication step leveraging duplex metabarcoding was applied to generate consensus sequences and reduce false positives. *Helicobacter pylori*-specific reads were identified using Kraken2 v1.2.2 [10] with the PlusPF database, which

includes bacterial, plasmid, viral, human, protozoa, and fungal sequences. These reads were then mapped to the *H. pylori* reference genome J99 (assembly ASM98269v1) [11] using Bowtie2 [12], and consensus sequences for all targeted regions were extracted with freebayes v1.3.6 [13] and bcftools v1.1.19 [14]. A custom suite of tools analyzed these consensus sequences to detect genomic markers and characterize the bacterial resistome. Simultaneously, a *de novo* assembly was performed using NCBI's SAUTE v2.5.1 [5] to obtain complete sequences for the *cagA* and *vacA* genes. Sequences for MLST were concatenated and analyzed using STRUCTURE v2.3.4 [7] to assign samples to annotated populations, which were compared to allele sequences in the PubMLST database [15]. Phylogenetic trees were constructed with RAxML-NG v1.2.2 [16] using the GTR + Gamma model. A set of custom scripts compiled the analysis results into comprehensive PDF and HTML reports, including circular genome representations generated with the Circos graphic library v0.69-9 [17].

Results

Clinical presentation

An 88-year-old woman was admitted to the emergency department of Lyon's public hospital on 23 September, 2023, with a left femoral neck fracture caused by a mechanical fall. Her medical history included permanent non-embolism IC/ACFA, toxic multinodular goiter treated with antithyroid drugs since 2018, and hypertension. Her surgical history consisted of partial hysterectomy in 1973, ovariectomies in 1974 and 1976, tonsillectomy, saphenous vein stripping in 2006, cataract surgery, colonoscopy, and facial fracture from a fall in 2021. Her alcohol consumption was moderate at one to two glasses of wine daily. Regular medications included apixaban, metoprolol, furosemide, digoxin, thiamazole, and sacubitril/valsartan.

Upon admission, the patient's C-reactive protein (CRP) level was within the reference range, with no evidence of an inflammatory syndrome. On 25 September, she underwent surgery to implant an intermediate left hip prosthesis. Postoperatively, the patient developed renal failure, likely due to dehydration. Transthoracic echocardiography revealed moderately impaired left ventricular systolic function (40–45%), normal global longitudinal strain, no pulmonary arterial hypertension, a dilated left atrium, and degenerative mitro-aortic changes. The postoperative course was complicated by deglobulation (hemoglobin: 7.9 g/dL), which was successfully managed with transfusion, and by a substantial hematoma near the surgical incision and within the thigh.

On 27 September, the patient developed a biological inflammatory syndrome, with a CRP level of 146 mg/L and leukocyte count of 14.2 G/L, although she exhibited no fever or signs of infection. The surgical scar remained clean and non-inflammatory, but a pressure sore developed on the left ankle without hypodermatitis. On 3 October, she was transferred to a rehabilitation unit. By 9 October, inflammation and citrine discharge were noted at the surgical site; however, there was no indication of prosthesis infection or osteitis, and the CRP level had declined to 39 mg/L.

On 12 October, blood cultures were taken as part of a routine infection assessment for the prosthesis. After 94 h of incubation, one aerobic bottle (Bact/Alert FA+; bioMérieux) yielded pleomorphic Gram-negative bacilli. The microorganism grew on blood agar under aerobic and microaerobic conditions within 3 days. Identification by matrix-assisted laser desorption/ionization time-of-flight (Vitek-MS; bioMérieux) confirmed *H. pylori* (score: 99.9%), further verified by *H. pylori*-specific PCR (Ridagene; r-biopharm, Darmstadt, Germany) and 16 S rDNA PCR (Nanopore MinION; Oxford Nanopore, Oxford, UK). The strain was sent to the French Reference Centre for confirmation and expert opinion. Repeated blood cultures were sterile.

The patient was treated with cefotaxime for 7 days, and a gastroscopy on 27 October revealed inflammatory antritis without ulceration or a clear portal of entry. Biopsies showed chronic antritis with mild atrophy, intestinal metaplasia without dysplasia, and active fundic gastritis. Triple therapy with amoxicillin, clarithromycin, and a proton pump inhibitor was initiated on 3 November for 10 days.

On 2 November, the patient underwent angioplasty and stenting of the left upper femoral artery for critical ischemia. By 4 November, vascular purpura had developed on her legs and hands, prompting transfer to the internal medicine department for further investigation. Suspected toxic purpura led to discontinuation of amoxicillin and clarithromycin on 8 November. Despite borderline *H. pylori* serology (IgG: 0.9) (ELFA, Vidas; bioMérieux), eradication therapy with Pylera® (bismuth subcitrate potassium and metronidazole) and omeprazole was initiated on 29 November.

The patient developed multiple complications involving the heart, lungs (nosocomial SARS-CoV-2 infection, recurrent pleurisy), kidneys, digestive system (gastric hemorrhage), vasculature, and skin. She died on 29 April 2024.

NGS analyses

The NRCCH was tasked with confirming and further investigating this case using multiple molecular approaches. The strain isolated from the blood underwent NGS and was analyzed using NRCCH's in-house pipeline, as outlined in the Materials and Methods section. Identification by NGS

Table 2 Mean depth and coverage of antimicrobial resistance marker sites obtained through target enrichment followed by NGS on DNA extracted from FFPE gastric biopsy

Gene	Mean depth	5× coverage (%)
16 S rDNA	52.5	100
23 S rDNA	64.4	100
gyrA	7.1	70
rpoB	10.2	90
rdxA	8.3	90
frxA	7.0	70
fdxB	9.8	90
cagA	7.6	80
vacA	8.2	70
pbp1	9.1	90
gyrB	9.3	80
yphC	8.5	70
ppa	12.1	100
htrA	12.1	90
efp	12.1	100
mutY	8.0	100
ureI	9.5	90
trpC	4.6	50
atpA	9.1	90
hsp60	11.6	90
All targets	14.1	90

Observed mean depth and coverage were calculated with a minimum threshold of 5-fold base depth across all coding sequences of the targeted genes, except for those defining the multilocus sequence typing scheme (*atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, and *yphC*), where only the shorter typing regions were considered for analysis

was confirmed with an average nucleotide identity score of >95% in favor of *H. pylori.*

The genome was fully sequenced and assembled, and it exhibited the following characteristics: 1,591,637 bp, mean depth of 471× coverage, 49 contigs (mean size: 32,482 bp), 1,484 coding sequences, and a GC content of 38.2%. The strain was identified as a hyper-virulent *cagA*-positive strain (*cagA*, EPIYA ABC) with a *vacA* genotype of s1i1m1. The resistome analysis revealed no resistance markers, aligning with the strain's fully susceptible profile observed in vitro.

The DNA extracted from the FFPE block sent to the NRCCH was analyzed via NGS using the target enrichment technique (see Materials and Methods). The reconstructed sequences were compared to those obtained by NGS from the strain isolated from the blood culture. Target enrichment on the FFPE biopsy yielded reconstructed sequences with an average coverage of 90% and an average depth of 14.1 (Table 2; Fig. 1).

The resistome identified using target enrichment on the FFPE biopsy was identical to that obtained from sequencing the genomic DNA of the blood culture strain. Similarly, the MLST classification confirmed that the strain belonged to the HpEurope group.

Conclusion

The *H. pylori* strain isolated from the blood culture bottle was likely identical to that identified via target enrichment on the FFPE biopsy. The gastric portal of entry is the most probable route of translocation, although no evidence explains this occurrence. The strain's hyper-virulent profile (*cagA*-positive and *vacA* s1i1m1) is not a recognized factor that predisposes to gastric translocation. The effectiveness of the probabilistic Pylera® regimen used for eradication in this case remains uncertain.

As described in previous publications [1, 2], *H. pylori* bacteremia often lacks severe clinical manifestations, consistent with the case presented here. The bacteriology laboratory faced no difficulties culturing *H. pylori* from the blood culture bottles despite the long positivity time (94 h), which is within the routine incubation period of up to 5 days. Extending the blood culture incubation period upon clinician request, when feasible, may increase the detection rate. Additionally, positive cultures could benefit from combined approaches, including PCR amplification followed by pan-bacterial *16 S rDNA* sequencing and, where available, metagenomics or NGS, as demonstrated in this study and others [3].

The target enrichment approach described for FFPE biopsies is novel and has not been previously reported. This promising strategy is currently under evaluation at our expert center and holds potential for broader application in the future.

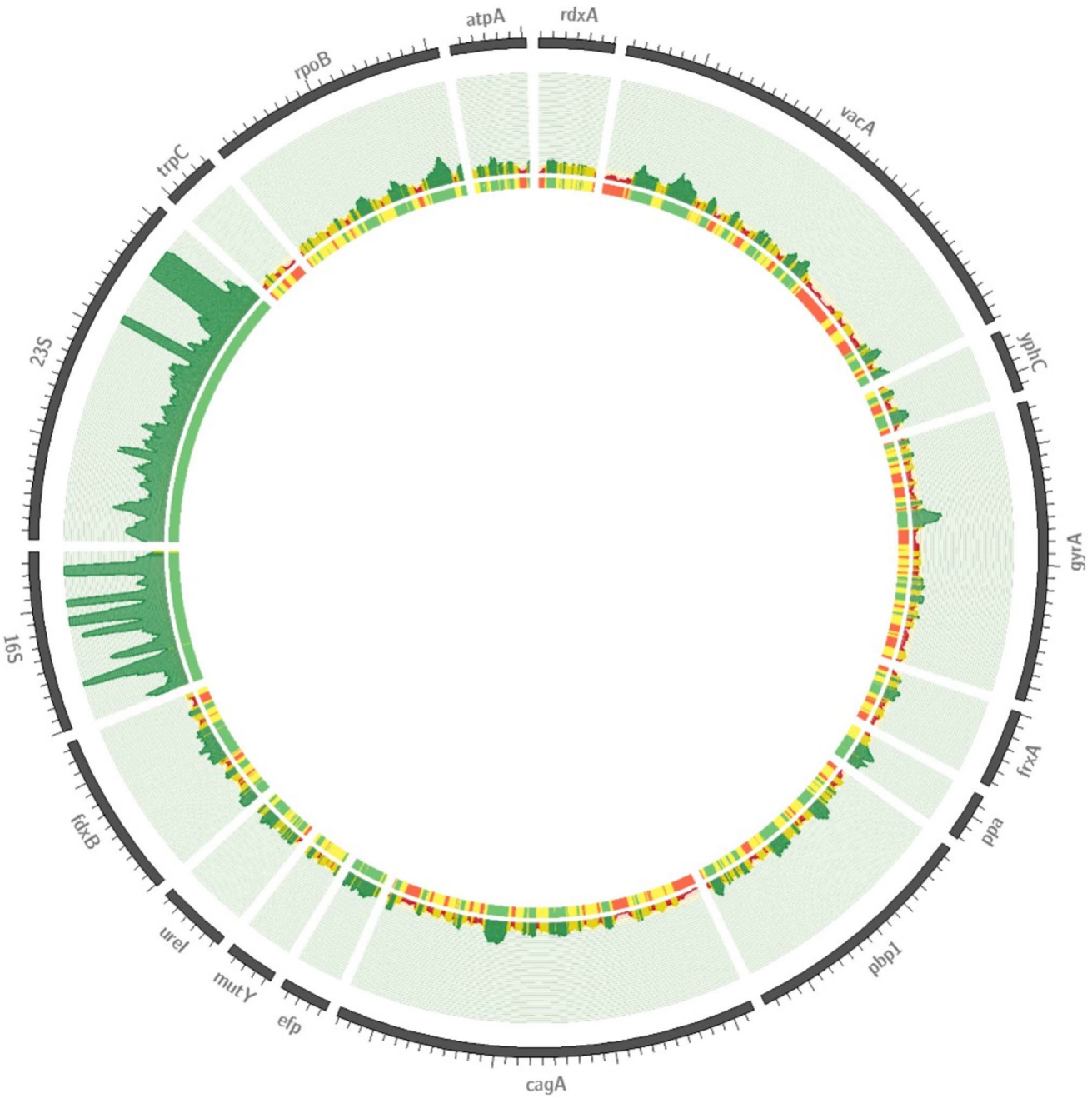

Fig. 1 Circular representation of sequencing results obtained through target enrichment followed by NGS on DNA extracted from the FFPE gastric biopsy

The overall sequencing quality of all targeted regions is depicted using a circular representation. Fold coverage depth is color-coded as follows: green for positions with a depth of >10-fold, yellow for positions with a depth between 5- and 10-fold, and red for positions with a depth of <5-fold. Drops in depth are expected in regions where the sample's genotype differs from the J99 reference strain genotype (i.e., *vacA*, *cagA*)

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Author contributions PL supervised the study. LB, MJ, LG, QJ, AGR, CS and PL analyzed the data and drafted the paper. CP, AD, JA and TG performed the experiments. All authors interpreted the data. All authors critically revised the manuscript for important intellectual content.

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Data availability The data that support the findings of this study are openly available in NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/bioproject/, reference number PRJNA1190696.

Declarations

Human ethics and consent to participate All diagnostic methods were performed as part of routine clinical care. Remnants of specimens were preserved at the Centre de Ressources Biologiques-Bordeaux Biothèque Santé of Bordeaux University Hospital under contract number 17DMIP017_6 with no information regarding patient identity. All patient data were anonymously reported.

Consent for publication The patient provided written informed consent for the publication of this article.

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that they have no conflicts of interest.

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