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RESEARCH

The first two human infections with *Helicobacter zhangjianzhongii*, a new *Helicobacter* closely related to *Helicobacter canis*

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Abstract

Purpose In 2023, *Helicobacter zhangjianzhongii* was proposed as a new species in the *Helicobacter* genus. We here describe two human cases of *H. zhangjianzhongii* bacteremia.

Methods Four clinical strains from the *Helicobacter* genus isolated from blood culture between 2017 and 2023 were studied. They were initially identified as *H. canis* by MALDI-TOF and 16S rDNA sequencing. The strains were biochemically characterized and tested at different temperatures and atmospheres. Two databases were used to characterize the isolates: the Bruker® MBT compass Version 4.1.1 database and a in-house spectrum-enriched database. After bacterial DNA extraction the genomes were sequenced on NovaSeq 6000 (Illumina) and analyzed using an in-house pipeline.

Results Case 1 involved a 58-year-old woman who was hospitalized in a thoracic oncology unit because her general condition deteriorated in a setting of small-cell carcinoma. She presented with abdominal pain associated with significant hepatomegaly. Case 2 involved a 78-year-old woman on rituximab who was hospitalized to treat chest pain, anemia, and inflammatory syndrome. Both strains exhibited very similar microbiological and genomic characteristics, thus growth in a microaerobic atmosphere at 37°C and 42°C, oxidase-positivity, and urease- and catalase-negativity. Both were formally identified by whole-genome sequencing as *H. zhangjianzhongii* (ANI > 99% and DDH > 94%).

Conclusion This proposed species is associated with bacteremia in humans. It is thus likely to be a novel human pathogen. Dogs may have been the source of infection.

Keywords MALDI-TOF · NGS · *Helicobacter* · New species · Zoonosis

Introduction

Bacteria of the *Helicobacter* genus are currently classified into two main groups depending on their preferred niches: gastric and enterohepatic Helicobacters. Enterohepatic Helicobacters account for two-thirds of all species in the *Helicobacter* genus. They generally colonize the small intestine, cecum, colon, rectum, and sometimes the liver, although some species are also found in the stomach. To date, 34 species have been identified, of which the most recent is *H. ibis* [1].

Many *Helicobacter* spp. are found in domestic animals such as dogs and cats. It is therefore likely that these animals may transmit the bacteria to humans. The pathogenic roles played by enterohepatic Helicobacters have been well-described in humans [2]. Of these Helicobacters, *H. cinaedi*

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is the most frequently found in humans, with increasing numbers of cases in recent decades. *H. cinaedi* can cause bacteremia that is frequently associated with cellulitis. Immunocompromised patients are more at risk than others [3–13], although immunocompetent individuals can also be infected [8, 14]. *H. fennelliae* has also been associated with human bacteremia. At least 25 cases have been described in the literature [15, 16]. Almost all patients had underlying immunodepression. One case of *H. canadensis* bacteremia has been described in an immunocompetent 35-year-old man [6]. PCR tests of stool or biopsy samples from patients with inflammatory bowel disease (IBD) have revealed *H. pullorum, H. cholecystus, H. canadensis, H. hepaticus, H. trogontum, H. bilis, H. fennelliae, H. mustelae, H. canis, H. ganmani, H. cinaedi, H. muridarum,* and *H. brantae* [17–21], although causal links with the inflammatory pathologies remain to be demonstrated [22, 23].

Certain enterohepatic *Helicobacter* species, including *H. bilis, H. ganmani, H. hepaticus,* and *H. pullorum*, are associated with hepatobiliary malignancies. Several groups have suggested that *H. bilis* infections are linked to biliary tract and gallbladder cancers of at-risk populations, thus Japanese, Thai, and Chilean individuals [24–29]. *H. bilis* may also be involved in the carcinogenesis of certain colorectal cancers [30]. *H. pullorum* has been associated with hepatobiliary and autoimmune digestive pathologies, and is also one of the most common *Helicobacter* species associated with gastroenteritis. Many cases have been described in the literature [31, 32]. However, *H. pullorum* has been detected both in asymptomatic patients and those with gastroenteritis, at rates of 4% and 4.3% respectively [33]. This implies that *H. pullorum* in the gastrointestinal tract is not necessarily pathogenic.

Stanley et al. [34] were the first to isolate and describe *H. canis* from dog feces. The helical or rod-shaped Gram-negative bacillus bears a sheathed flagellum at either end. This enterohepatic *Helicobacter* is a rare human pathogen that causes principally bacteremia, sometimes associated with skin involvement, and mainly affects immunocompromised patients [35, 36]. It has also been found in IBD patients. Although the mode of transmission has not yet been clearly established, it would appear that domestic animals, mainly cats and dogs, are often associated with human infections.

Helicobacter zhangjianzhongii has more recently been proposed as a new species [37] that is closely related to *H. canis*. It is also a Gram-negative helical bacillus with a sheathed flagellum at either end. It is oxidase-positive and catalase- and urease-negative. This species was also initially isolated from dog feces. The National Reference Center for Campylobacters and Helicobacters (NRCCH) has received two *Helicobacter* isolates that resemble *H. canis*. These came from two bacteremia cases, one of which was identified in 2017 at Bordeaux University Hospital and the other in 2023 at Chambéry Hospital. This study describes the biochemical, phenotypic, and genomic features that enabled explicit identification of the first two human cases of *H. zhangianzhonii* infection. We also present the clinical contexts of both cases of bacteremia.

Materials and methods

Strains studied

Four clinical *Helicobacter* strains isolated from blood cultures between 2017 and 2023 were studied (Table 1). They were initially identified as *H. canis* by MALDI-TOF and *16S rDNA* sequencing. The strains were named 2017-C, 2023-V, 2020-A and 2022-F.

Biochemical and phenotypic characterization

All four strains were biochemically characterized using the API CAMPY (bioMérieux) galleries as recommended by the supplier. The enzymatic component of the gallery was incubated for 24 h at 37°C in an aerobic atmosphere. The other part of the gallery was incubated for 48 h at 37°C in a microaerobic atmosphere. The following enzyme activities were measured: urease, nitrate reductase, esterase, hippuricase, ɣ-glutamyl transpeptidase, triphenyltetrazolium chloride reductase, pyrrolidonyl arylamidase, L-arginine arylamidase, L-aspartate arylamidase, and alkaline phosphatase. H_2S production was tested, as was assimilation of the following compounds: glucose, sodium succinate, sodium acetate, sodium propionate, malate, and citrate trisodium. Oxidase status was assessed using a BBLTM Dryslide membrane (Becton Dickinson, Franklin Lakes, NJ, USA) and catalase status employing 30 volumes of hydrogen peroxide. Gram-staining was systematically performed using an Aerospray GRAM model 7322 device (ELITechGroup, Puteaux, France).

Culture conditions

The growth of all four strains was tested at 25°C, 37°C, and 42°C. Agar plates were incubated for 48 h. Bacterial growth was systematically checked after 24 h of incubation. During growth at 25°C or 42°C, a control was systematically cultured at 37°C in parallel. To study phenotypic characteristics, the strains were plated on in-house Pylo agar and Columbia blood agar (CBA) (ThermoFisher Scientific, Waltham, MA, USA) with 5% (v/v) sheep blood. The selective Pylo agar is manufactured by the NRCCH and features a Wilkins Chalgren base, Vancomycin 10 g/L, Amphotericin B 1 g/L, Cefsulodine 2 g/L, Trimethoprim 5 g/L, and 10% (v/v) human blood.

Microaerobic culture proceeded in a BAKER Ruskinn Concept chamber under 85% N_2, 10% CO_2 and 5% O_2, all

Table 1 *Helicobacter zhangjianzhongii* and *Helicobacter canis* strains included in this study

Name	Species	Reference	Source	Origin (country, town)
2017-C	*zhangjianzhongii*		Blood (human)	France, Bordeaux
2023-V	*zhangjianzhongii*		Blood (human)	France, Chambéry
2020-A	*canis*		Blood (human)	France, Indre
2022-F	*canis*		Blood (human)	France, La Rochelle
CCUG-32756 T	*canis*	REF-32756 T		Suisse, Berne
NCTC-12740 (NCBI)	*canis*	REF-12740	(human)	Suisse, Berne
CPD2-1	*zhangjianzhongii*	REF-CPD2-1	Stools (dog)	China, Beijing
XJK30-2	*zhangjianzhongii*	REF-XJK30-2	Stools (dog)	China, Beijing

v/v. A Thermo Scientific Sachet Oxoid AnaeroGen device was used to create anaerobic conditions. During all aerobic and anaerobic incubations, microaerobic growth controls were systematically established in parallel. All bacterial cultures were checked at 24 h and 48 h.

MALDI-TOF

Two databases were used to characterize the isolates. These were, first, the Bruker MBT compass ver. 4.1.1 database of Bordeaux University Hospital, which contains 24 spectra of *Helicobacte*r spp., including 2 of *H. canis* (*H. canis* ZC80F-NVU and *H. canis* CCUG-32756), and, second, a spectrum-enriched NRCCH database. The latter contains nine NRCCH spectra of nine species of *Helicobacter*. The protein spectra of different strains can thus be compared. Main spectrum profiles (MSPs) were generated using flexControl ver. 3.4 (Bruker Daltonics) and MBT Compass Explorer ver. 4.1.100 (Bruker Daltonics, Billerica, MA)The method has been described by Spergser et al. [38]. Twenty-four mass spectra were obtained for each strain using flexControl software. A minimum of 18 mass spectra were used to create each MSP.

NGS and bioinformatic analyses

Bacterial DNA extraction employed the MagNA Pure 96 system (Roche Diagnostics, Meylan, France), MagNA Pure 96 DNA, and the Viral NA Small Volume kit. Quantification and purity, thus the 260-/280-nm and 260-/230-nm ratios, were determined spectrophotometrically (DeNovix, Wilmington, DE). An iSeq 100 (Illumina, San Diego, CA) sequencer was used to analyze isolates 2017-C, 2020-A and 2022-F and a NovaSeq 6000 (Illumina) sequencer when studying isolate 2023-V. After sequencing, FastQC ver. 0.11.8 software [39] was used for quality assurance and Sickle ver. 1.33 [40] software to eliminate poor-quality reads. All reads were assembled with the aid of SKESA ver. 2.5.1 software [41]. Contigs smaller than 200 base pairs and those with read depths < 5 were eliminated using an in-house Python script. A homemade pipeline was used to identify resistance and virulence genes. The pipeline features the Nucleotide-Nucleotide/Protein–Protein BLAST 2.12.0 + online tool [42] and NCBI, Card, Resfinder, Plasmidfinder, and in-house databases. Phylogenetic trees were constructed using the GyrA protein and *16S rDNA* sequences: Muscle ver. 3.8.1551 [43] software was used for alignment and the neighbor-joining method of Mega ver. 11.0.13 software [44] was employed to calculate phylogenetic distances. This featured 1,000 replications using the Complete-deletion calculation option of the Kimura-2 parameter method. All phylogenetic trees were drawn with the aid of iTOL ver. 5 [45]. The mathematical models of the ANI and DDH indices were used to compare the genomes to those in "public" databases. The ANI was calculated using FastANI ver. 1.1 [46] and the DDH using the online tool TYGS ver. 391 (GGDC) [47]. The results were visualized using the Python seaborn module ver. 0.12.2. The between-genome scores were > 95% for the ANI and > 70% for the DDH.

The genomic data on two strains of the newly published *H. zhangjianzhongii* species were retrieved from public databases. Other information, including the biochemical and phenotypic characteristics, and the origins, was obtained from the literature [37].

Data availability

The genomes are available as raw read files under ENA project accession number PRJEB82765 and read accession numbers ERR13992622, ERR13992623, ERR13992624, and ERR13992625 for 2023-V, 2020-A, 2017-C, and 2022-F, respectively.

Results

Biochemical and phenotypic characterization

All four strains were negative for urease, catalase, nitrate reduction, and hippurate hydrolysis. All were positive on the oxidase, alkaline phosphatase, and esterase tests (Table 2).

Table 2 Microbiological characteristics of *Helicobacter canis* and *Helicobacter zhangjianzhongii*

	2017-C	2023-V	2020-A	2022-F	*H. canis* REF-32756T	*H. canis* REF-12740	*H. zhangjian-zhongii* REF-CPD2-1	*H. zhangji-anzhongii* REF-XJK30-2
Urease	-	-	-	-	-	-	-	-
Oxidase production	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-
Nitrate reductase	-	-	-	-	-	-	-	-
PAL	+	+	+	+	+	+	+	+
Esterase	+	+	+	+	"±"	"±"	+	+
GGT	+	+	+	+	+	Unknown	+	+
Hippuricase	-	-	-	-	-	-	-	-
PyrA	-	-	-	-	Unknown	Unknown	+	+
ArgA	-	-	-	-	Unknown	Unknown	+	+
AspA	-	-	-	-	Unknown	Unknown	+	+
TTC	-	-	-	-			+	+
H_2S	-	-	-	-			-	-
No. of flagella	Unknown	Unknown	2	Unknown	2	2	2	2
Sheathed flagella	Unknown	Unknown	+	Unknown	+	+	Unknown	Unknown
Flagellar disposition	Unknown	Unknown	Bipolar	Unknown	Bipolar	Bipolar	Bipolar	Bipolar
Microaerobic growth, 25 C	-	-	-	-	-	-	-	-
Microaerobic growth, 37°C	+	+	+	+	+	+	+	+
Microaerobic growth, 42°C	+	+	-	+	+	+	+	+
Anaerobic growth, 37°C	-		-	-	-	-	-	-
Aerobic growth, 37°C	-		-	-	-	-	-	-

All exhibited gamma-glutamyl transpeptidase activity. The two strains recently described as *H. zhangjianzhongii*, thus REF-CPD2-1 and REF-XJK30-2, were reported to exhibit pyrrolidonyl arylamidase, L-arginine arylamidase, L-aspartate arylamidase, and TTC reductase activities. None of the four strains grew under anaerobic conditions at 37°C, under aerobic conditions at 37°C, or under microaerobic conditions at room temperature. However, all grew under microaerobic conditions at 37°C and all except 2020-A under microaerobic conditions at 42°C.

MALDI-TOF

The Bruker database did not allow identification of 2017-C and 2023-V to species (Table 3). However, the MSPs of strains 2020-A and 2022-F matched that of one *H. canis*, *H. canis* ZC80F NVU, well in the Bruker database. The score was > 1.7. The NRCCH database yielded a score > 2 when the MSP of strain 2022-F was compared to the MSP of strain 2020-A, and vice versa. Similarly, a score > 2 was obtained when the MSP of strain 2017-C was compared to the MSP of strain 2023-V, and vice versa (Table 3).

Table 3 MALDI-TOF identification of the 2017-C, 2023-V, 2020-A, and 2022-F strains, and two *Helicobacter canis* reference strains, and the scores obtained using the Bruker® and NRCCH enriched databases

MSP	Bruker® database	Score	NRCCH database	Score
2022-F	*H. canis* ZC80F NVU	1.710	2020-A	2.470
2020-A	*H. canis* ZC80F NVU	1.760	2022-F	2.360
2023-V	No match	/	2017-C	2.170
2017-C	No match	/	2023-V	2.320

Only scores > 1.7 are shown

NGS

Phylogenetic analyses of trees based on the gene encoding the GyrA protein, and 16S rDNA, confirmed that the strains belonged to the *Helicobacter* genus. The reference strains of *H. zhangjianzhongii* REF-CPD2-1 and REF-XJK30-2 were close to the reference strains of *H. canis* (Table 4). Within this subgroup, 16S rDNA-based phylogenetic tree analysis distinguished two distinct clusters of four strains

Table 4 Characteristics of the genomes analyzed in this study

Genome	No. of contigs	Genome size (bp)	Average contig size (bp)	GC%	No. of CDs
2022-F	77	2,047,299	26,588.3	44.47	1,948
2020-A	95	1,971,321	20,750.75	45.01	1,843
2023-V	70	2,434,004	34,771.49	44.5	2,200
2017-C	75	2,169,485	28,926.47	43.71	1,969
H. canis CCUG-32756 T	26	1,930,063	74,233.19	44.15	1,800
H. canis NCTC-12740	1	1,932,823	-	45	1,794
H. zhangjianzhongii CPD2-1	26	2,096,761	80,644.65	43.88	1,895
H. zhangjianzhongii XJK30-2	29	2,084,878	71,892.34	44.34	1,873

CDs: coding sequences

(Fig. 1). The first included the two reference strains of *H. canis* and the two clinical strains 2020-A and 2022-F. The second comprised two *H. zhangjianzhongii* strains and the clinical strains 2017-C and 2023-V. Notably, strain 2022-F seemed less well-affiliated with the cluster on the GyrA tree than were other strains (Fig. 2).

The ANI scores confirmed the results of *16S rDNA* sequence analysis. The comparative ANI scores of strains REF-CPD2-1, REF-XJK30-2, 2017-C, and 2023-V were all > 99%, indicating close phylogenetic proximity (Fig. 3). Within the second cluster of *H. ca*nis isolates—2020-A, 2022-F, CCUG-32756T and NCTC-12740, all ANI scores were > 95%. However, none of the four strains in the first cluster exhibited an ANI score > 95% when compared to any strain in the second cluster. There were thus two distinct species. The results obtained using the DDH tool corroborated these conclusions (Fig. 4). No resistance markers were identified in the resistomes of the strains (data not shown).

Discussion

We sought to characterize two strains of *Helicobacter* isolated from humans. Both were closely related to *H. canis*. We considered the recent discovery of a new, closely related species termed *H. zhangjianzhongii* [37]. We found that both isolates were indeed the newly proposed species. This is the first description of the pathogenicity thereof in humans.

The first strain, 2017-C, was isolated in Bordeaux from an aerobic blood culture bottle. A 58-year-old patient with disseminated small-cell bronchial carcinoma was undergoing chemotherapy. The time to positivity was 4 days. Given the prolonged apyrexia of the patient, no antibiotic treatment was initiated. The second strain, 2023-V, was isolated from two aerobic blood culture bottles. A 78-year-old woman was on long-term rituximab to treat neutrophilic, cytoplasmic antibody vasculitis. She was admitted to hospital with chest pain, anemia, and a spontaneously resolving 39°C fever. An inflammatory syndrome was detected. Her C-reactive protein level was 70 mg/L. The times to positivity of blood samples taken at admission were 87 h and 115 h. The inflammatory syndrome improved rapidly on prescription of amoxicillin + clavulanic acid.

Bioinformatics tools separated the eight compared genomes into two very distinct clusters. Strains REF-CPD2-1, REF-XJK30-2, 2017-C, and 2023-V were *H. zhangjianzhongii*. Strains 2020-A, 2022-F, CCUG-32756T, and NTCT-12740 were *H. canis*. The phenotypic and bacterial growth characteristics were rather similar. All strains were mobile, helical Gram-negative bacilli that grew at 37°C, and sometimes also at 42°C, under microaerobic conditions. The biochemical analyses were also identical. However, *H. zhangjianzhongii* CPD2-1 and XJK30 2 were positive for PyrA, ArgA, TTC, and AspA activities whereas the two clinical strains 2023-V and 2017-C were not. Heterogeneity in terms of enzymatic activities has been described well in the literature for other *Helicobacter* species [36, 48–51], perhaps reflecting environmental differences in terms of the host source and storage conditions. Interpretation bias may also be in play. Humans interpret color changes.

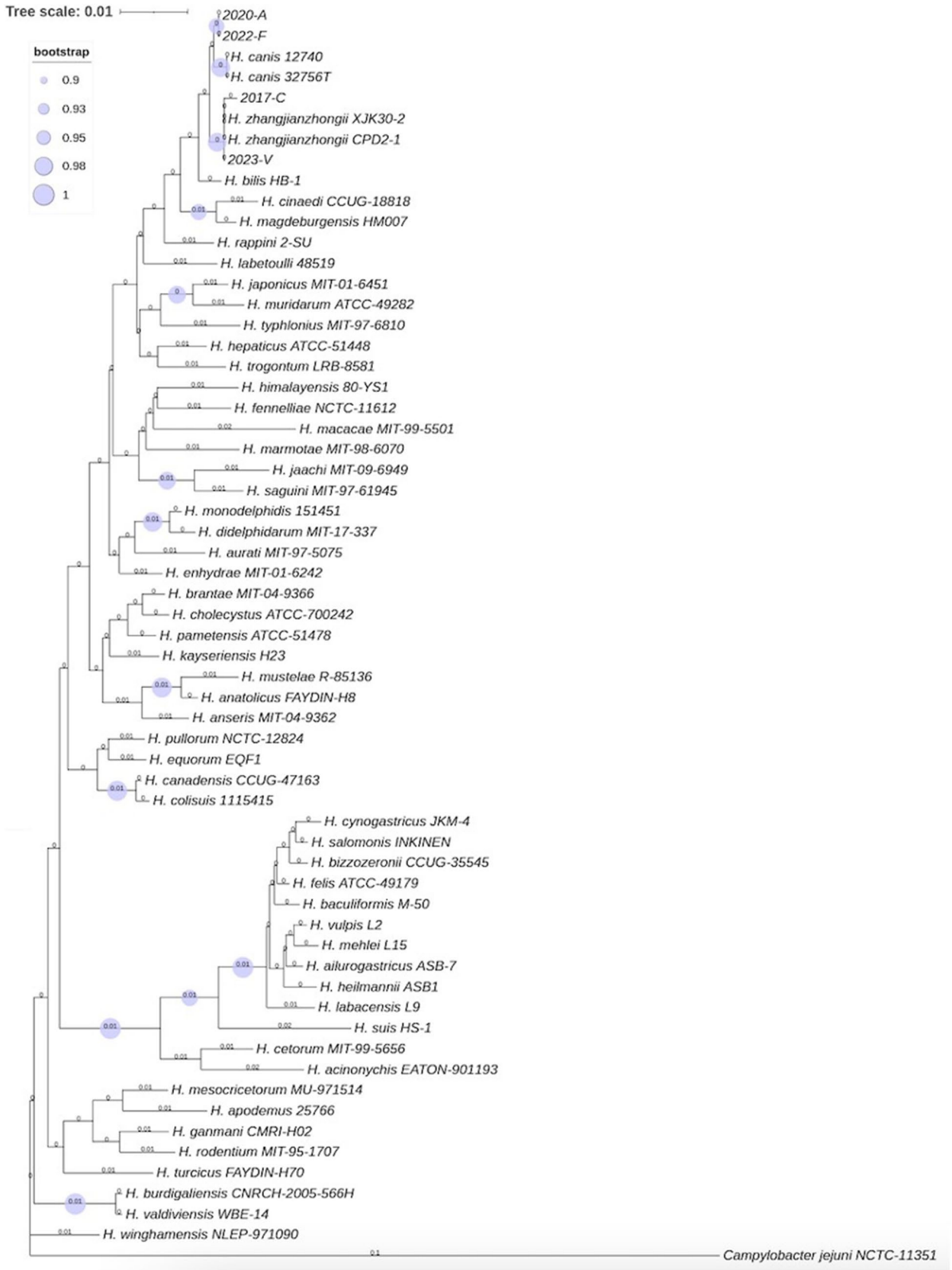

Fig. 1 Neighbor-joining phylogenetic tree based on the ***16S rDNA*** gene. *Campylobacter jejuni* served as the root group. Boostraps greater than 0.9 are shown by blue circles, the sizes of which are proportional to the values

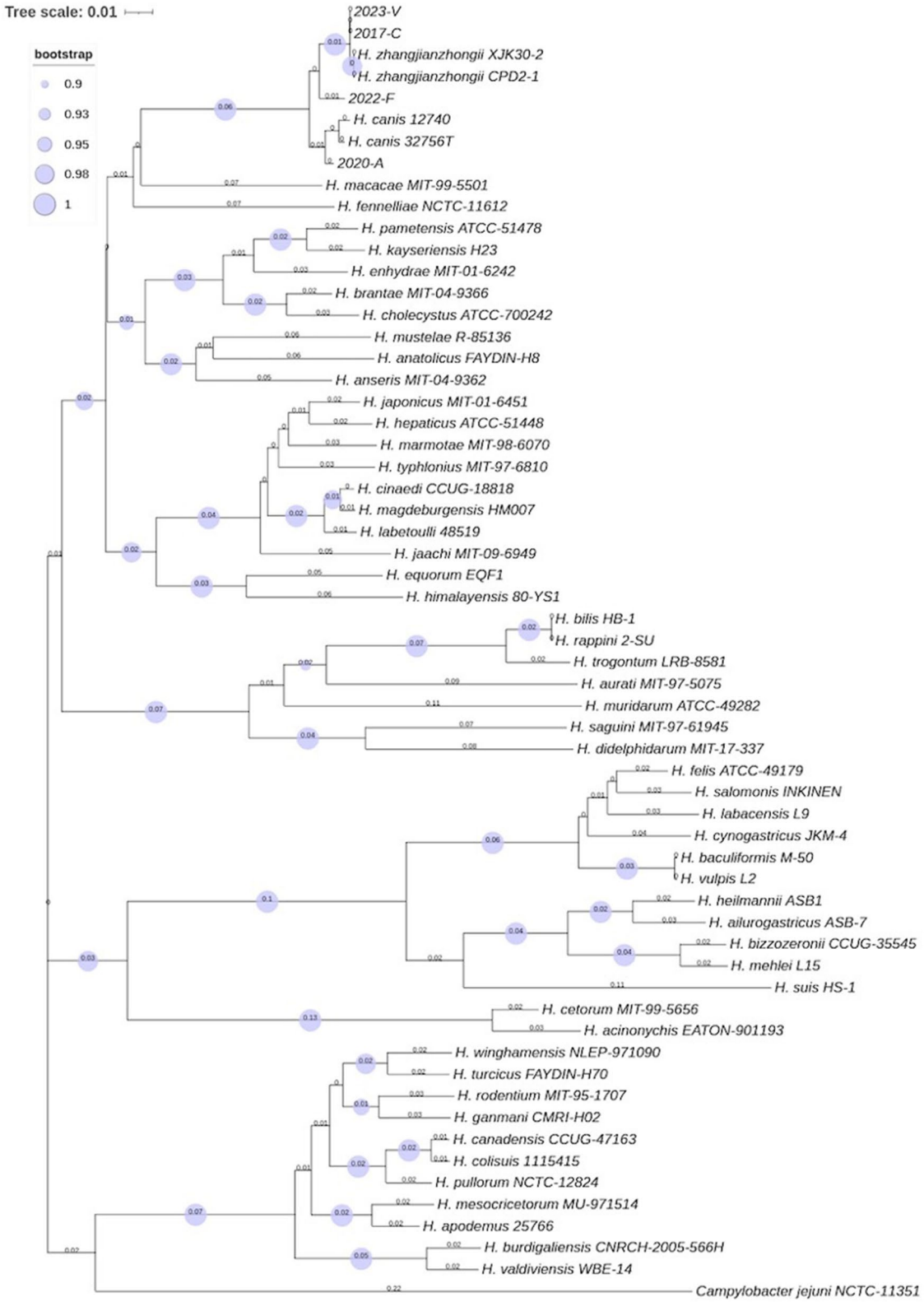

Fig. 2 Neighbor-joining phylogenetic tree based on GyrA. *C. jejuni* served as the root group. Boostraps greater than 0.9 are shown by the blue circles, the sizes of which are proportional to the values. A clustered heatmap based on the genomic ANI values of clinical and reference strains of *H. canis* and *H. zhangjianzhongii*. REF-12093, *Helicobacter pylori* JCM-12093; REF18818, *Helicobacter cinaedi* CCUG-18818; REF-XJK30-2, *Helicobacter zhangjianzhongii* XJK30-2; REF-CPD2-1, *Helicobacter zhangjianzhongii* CPD2-1; REF-32756T, *Helicobacter canis* CCUG-32756T; REF-12740, *Helicobacter canis* NCTC-12740

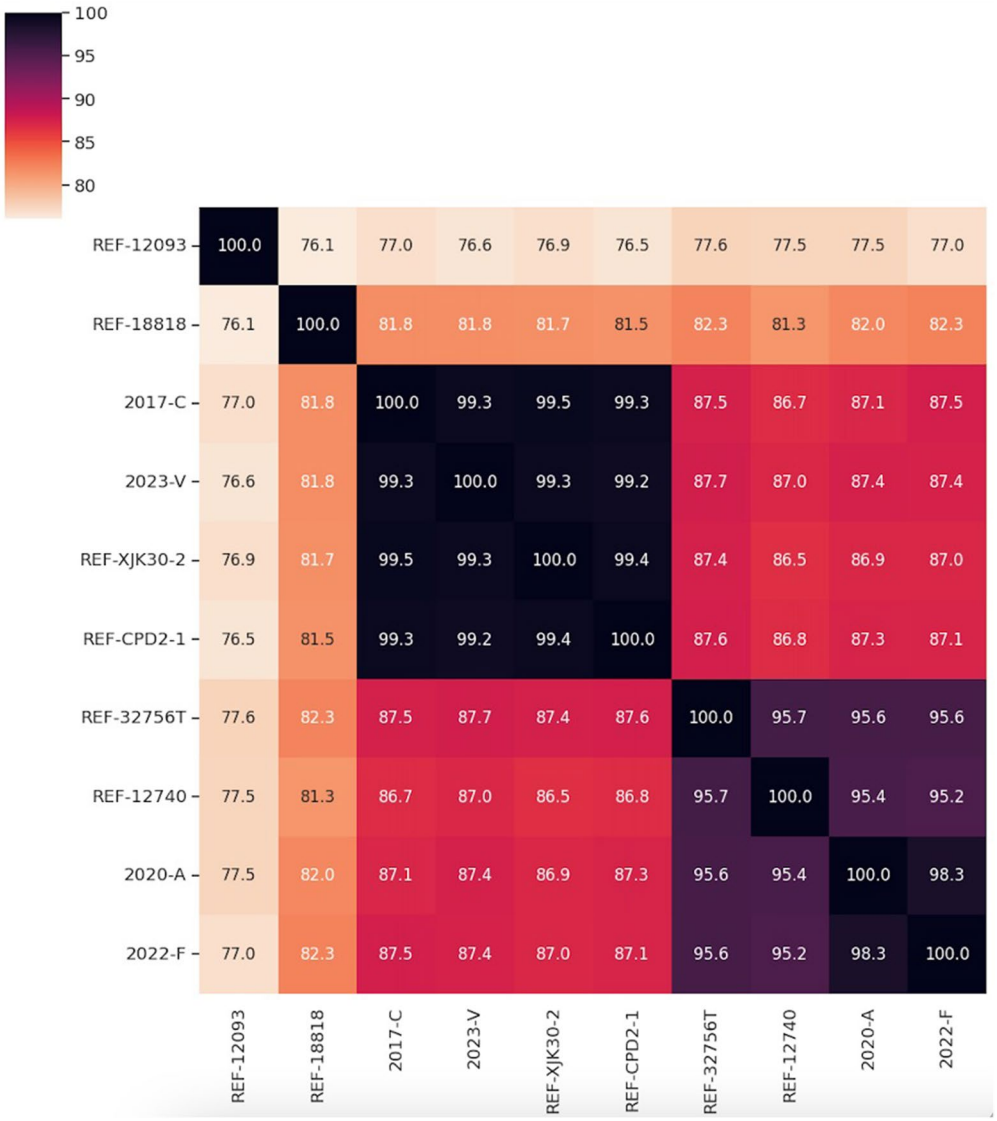

Fig. 3 A clustered heatmap based on the genomic ANI values of clinical and reference strains of *H. canis* and *H. zhangjianzhongii*. REF-12093, *Helicobacter pylori* JCM-12093; REF18818, *Helicobacter cinaedi* CCUG-18818; REF-XJK30-2, *Helicobacter zhangjianzhongii* XJK30-2; REF-CPD2-1, *Helicobacter zhangjianzhongii* CPD2-1; REF-32756T, *Helicobacter canis* CCUG-32756T; REF-12740, *Helicobacter canis* NCTC-12740

Resistome analysis did not reveal any resistance marker, consistent with microbial susceptibility to a cyclin, a β-lactam, macrolides, and fluoroquinolones. Also, the 78-year-old patient exhibited favorable clinico-biological progression on antibiotic treatment. The absence of any antimicrobial resistance marker in our strains and those already described implies that the reservoir(s) lack(s) any antibiotic selection pressure.

The MALDI-TOF results separate the four strains into two groups. On the one hand, strains 2023-V and 2017-C seemed more distant from the other strains, and, on the other hand, strains 2022-F and 2020-A, closer to, the MSP spectrum of *H. canis* ZC80F NVU strain in the Bruker database. However, strains 2022-F and 2020-A were not formally identified by that database as *H. canis*. This may be because the database lacks a sufficient number of MSPs, because different culture media were used [52, 53], or because protein expression varies greatly with the host, the environment, or/and transition to the coccoid form under certain conditions. Moreover, the MSPs of the two *H. canis* strains in the Bruker database did not match. The score was > 1.7 (data not shown). MALDI-TOF did not identify 2023-V or 2017-C as *H. zhangjianzhongii*. Neither spectrum was in the Bruker or in-house NRCCH database. The NRCCH has therefore updated the in-house database by renaming the MSPs of strains 2023-V and 2017-C, thus improving identification of the new species (scores > 2.1, Table 3).

H. zhangjianzhongii and *H. canis* were originally described from dogs [34, 37]. Although transmission from dog to human has not been proven, this seems not unlikely (36). We lacked information on whether dogs were present

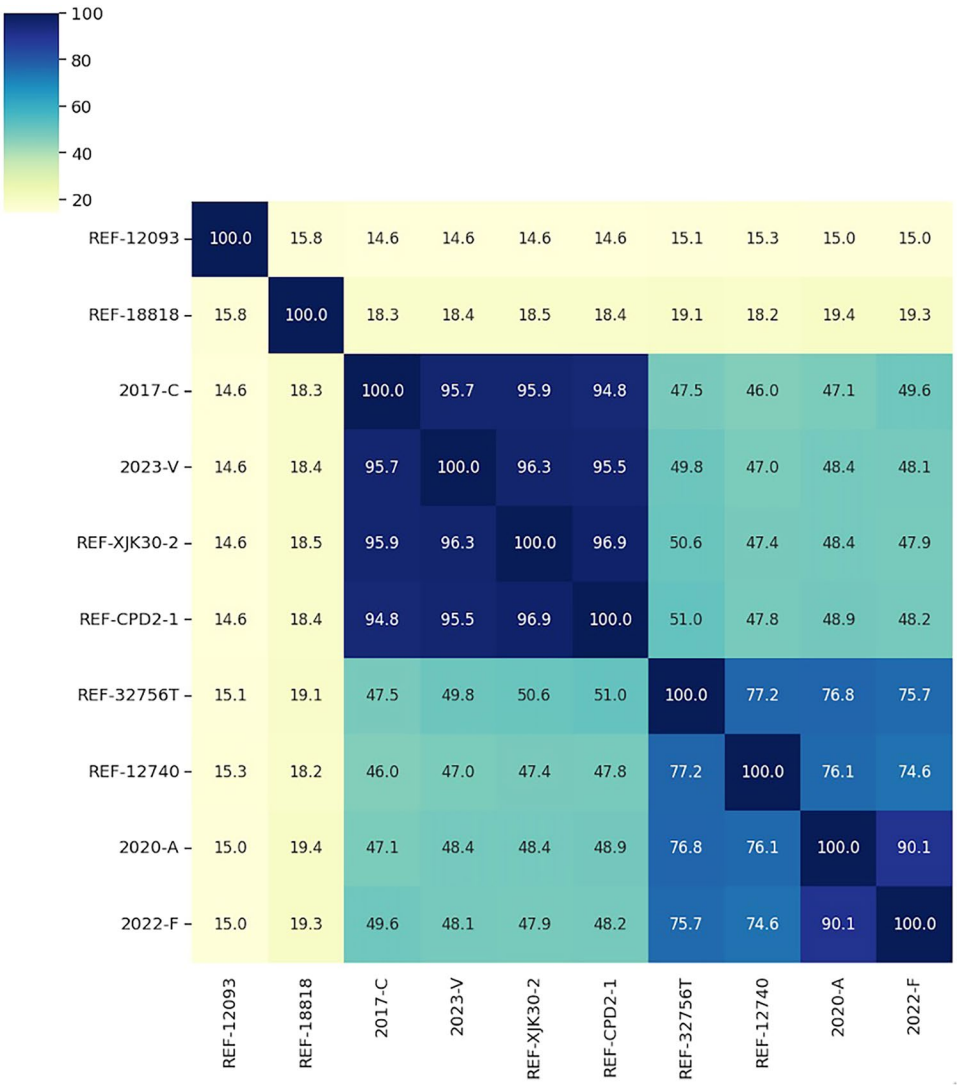

Fig. 4 A clustered heatmap based on the DDH genomic values of clinical and reference strains of *H. canis* and *H. zhangjianzhongii*. REF-12093, *Helicobacter pylori* JCM-12093; REF18818, *Helicobacter cinaedi* CCUG-18818; REF-XJK30-2, *Helicobacter zhangjianzhongii* XJK30-2; REF-CPD2-1, *Helicobacter zhangjianzhongii* CPD2-1; REF-32756T, *Helicobacter canis* CCUG-32756T; REF-12740, *Helicobacter canis* NCTC-12740

in the living environments of patients with *H. zhangjianzhongii* bacteremia.

Other NCBI *H. canis* genomes, notably NCTC-12410, were also studied in the course of this study. This strain, isolated from dog feces, exhibited ANI scores of 97.6% and 97.4% when compared to *H. zhangjianzhongii* XJK30 2 and CPD2 1 respectively. The ANI scores were 86.6% and 86.4% when NCTC-12410 was compared to the *H. canis* reference strains CCUG-32756T and NCTC-12740. The DDH analysis came to the same conclusion. The scores were 87.8% and 86.7% in comparisons with *H. zhangjianzhongii* XJK30 2 and CPD2 1 respectively, and 45.6% and 45.8% in comparisons with CCUG-32756T and NCTC-12740, respectively. The NCTC-12410 genome of the NCBI platform, identified as *H. canis*, should therefore be reclassified as *H. zhangjianzhongii*.

Conclusion

This study provides sufficient evidence to identify the first two cases of human infection with *H. zhangjianzhongii*, a new human pathogen. All *H. canis* identifications obtained using the current Bruker MALDI-TOF commercial database must be confirmed via NGS.

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Author contributions PL supervised the study. SW, QJ, LB, ML, MJ and PL analyzed the data and drafted the paper. SW, AD, and JA 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 European Nucleotide Archive (ENA) at EMBL-EBI (https://www.ebi.ac.uk/ena/browser/home). The ENA accession numbers are: ERR13992622 (2023-V), ERR13992623 (2020-A), ERR13992624 (2017-C), and ERR13992625 (2022-F).

Declarations

Conflict of interest The authors declare no competing interests.

Human ethics and consent to participate All diagnostic methods were routine. As the strains were sent to the NRCCH for research purposes, there was no need for ethical approval or informed patient consent. The need for scrutiny by an ethics committee was deemed unnecessary because the scientific mission of the national reference center is mandated by Santé Publique France (www.spf). All information that could possibly identify patients was removed.

Consent for publication Not applicable.

References

1. Lopez-Cantillo M, Vidal-Veuthey B, Mella A, de la Haba RR, Collado L (2023) *Helicobacter ibis* sp. nov., isolated from faecal droppings of black-faced ibis (Theristicus melanopis). Int J Syst Evol Microbiol. 73. https://doi.org/10.1099/ijsem.0.005983
2. Ménard A, Péré-Védrenne C, Haesebrouck F, Flahou B (2014) Gastric and enterohepatic helicobacters other than *Helicobacter pylori*. Helicobacter 19(Suppl 1):59–67. https://doi.org/10.1111/hel.12162
3. Kedra J, Zeller V, Heym B, Lehours P, Meyssonnier V, Lariven S et al (2018) A case of recurrent *Helicobacter cinaedi* prosthetic joint infection in an HIV-infected man. J Bone Jt Infect 3:230–233. https://doi.org/10.7150/jbji.28375
4. Araoka H, Baba M, Okada C, Kimura M, Sato T, Yatomi Y et al (2018) Risk factors for recurrent *Helicobacter cinaedi* bacteremia and the efficacy of selective digestive decontamination with kanamycin to prevent recurrence. Clin Infect Dis Off Publ Infect Dis Soc Am 1(67):573–578. https://doi.org/10.1093/cid/ciy114
5. Kiehlbauch JA, Tauxe RV, Baker CN, Wachsmuth IK (1994) *Helicobacter cinaedi*-associated bacteremia and cellulitis in immunocompromised patients. Ann Intern Med 15(121):90–93. https://doi.org/10.7326/0003-4819-121-2-199407150-00002
6. Tee W, Montgomery J, Dyall-Smith M (2001) Bacteremia caused by a *Helicobacter pullorum*-like organism. Clin Infect Dis Off Publ Infect Dis Soc Am 15(33):1789–1791. https://doi.org/10.1086/323983
7. Cimolai N, Gill MJ, Jones A, Flores B, Stamm WE, Laurie W et al (1987) "*Campylobacter cinaedi*" bacteremia: case report and laboratory findings. J Clin Microbiol 25:942–943. https://doi.org/10.1128/jcm.25.5.942-943.1987
8. Lasry S, Simon J, Marais A, Pouchot J, Vinceneux P, Boussougant Y (2000) *Helicobacter cinaedi* septic arthritis and bacteremia in an immunocompetent patient. Clin Infect Dis Off Publ Infect Dis Soc Am 31:201–202. https://doi.org/10.1086/313930
9. Pasternak J, Bolivar R, Hopfer RL, Fainstein V, Mills K, Rios A et al (1984) Bacteremia caused by Campylobacter-like organisms in two male homosexuals. Ann Intern Med 101:339–341. https://doi.org/10.7326/0003-4819-101-3-339
10. Matsumoto T, Goto M, Murakami H, Tanaka T, Nishiyama H, Ono E et al (2007) Multicenter study to evaluate bloodstream infection by *Helicobacter cinaedi* in Japan. J Clin Microbiol 45:2853–2857. https://doi.org/10.1128/JCM.00465-07
11. Rimbara E, Mori S, Kim H, Matsui M, Suzuki S, Takahashi S et al (2013) *Helicobacter cinaedi* and *Helicobacter fennelliae* transmission in a hospital from 2008 to 2012. J Clin Microbiol 51:2439–2442. https://doi.org/10.1128/JCM.01035-13
12. Aboulafia D, Mathisen G, Mitsuyasu R (1991) Aggressive Kaposi's sarcoma and campylobacter bacteremia in a female with transfusion associated AIDS. Am J Med Sci 301:256–258. https://doi.org/10.1097/00000441-199104000-00005
13. Roupie A-L, Lafont E, Fraitag S, Ferroni A, Lécuyer H, Boccara O et al (2023) Recurrent cellulitis revealing *Helicobacter cinaedi* in patient on ibrutinib therapy. France Emerg Infect Dis 29:640–641. https://doi.org/10.3201/eid2903.221329
14. Sato H, Ogino A, Matsuzaka S, Asami Y, Kanbayashi S, Masuda M et al (2019) Ovarian abscess caused by *Helicobacter cinaedi* in a patient with endometriosis. IDCases 17:e00578. https://doi.org/10.1016/j.idcr.2019.e00578
15. Fujiya Y, Nagamatsu M, Tomida J, Kawamura Y, Yamamoto K, Mawatari M et al (2016) Successful treatment of recurrent *Helicobacter fennelliae* bacteraemia by selective digestive decontamination with kanamycin in a lung cancer patient receiving chemotherapy. JMM Case Rep 3:e005069. https://doi.org/10.1099/jmmcr.0.005069
16. Saito S, Tsukahara M, Ohkusu K, Kurai H (2016) *Helicobacter fennelliae B*acteremia: three case reports and literature review. Medicine (Baltimore) 95:e3556. https://doi.org/10.1097/MD.0000000000003556
17. Kaakoush NO, Holmes J, Octavia S, Man SM, Zhang L, Castaño-Rodríguez N et al (2010) Detection of *Helicobacteraceae* in intestinal biopsies of children with Crohn's disease. Helicobacter 15:549–557. https://doi.org/10.1111/j.1523-5378.2010.00792.x
18. Bohr URM, Glasbrenner B, Primus A, Zagoura A, Wex T, Malfertheiner P (2004) Identification of enterohepatic *Helicobacter* species in patients suffering from inflammatory bowel disease. J Clin Microbiol 42:2766–2768. https://doi.org/10.1128/JCM.42.6.2766-2768.2004
19. Man SM, Zhang L, Day AS, Leach S, Mitchell H (2008) Detection of enterohepatic and gastric helicobacter species in fecal specimens of children with Crohn's disease. Helicobacter 13:234–238. https://doi.org/10.1111/j.1523-5378.2008.00607.x
20. Thomson JM, Hansen R, Berry SH, Hope ME, Murray GI, Mukhopadhya I et al (2011) Enterohepatic helicobacter in ulcerative colitis: potential pathogenic entities? PLoS ONE 23(6):e17184. https://doi.org/10.1371/journal.pone.0017184
21. Laharie D, Asencio C, Asselineau J, Bulois P, Bourreille A, Moreau J et al (2009) Association between entero-hepatic *Helicobacter* species and Crohn's disease: a prospective cross-sectional study. Aliment Pharmacol Ther 30:283–293. https://doi.org/10.1111/j.1365-2036.2009.04034.x
22. Yu Q, Zhang S, Li L, Xiong L, Chao K, Zhong B et al (2015) Enterohepatic *Helicobacter* species as a potential causative factor in inflammatory bowel disease. Medicine (Baltimore) 13(94):e1773. https://doi.org/10.1097/MD.0000000000001773
23. Yu Q, Zhang S, Li L, Xiong L, Chao K, Zhong B et al (2015) Enterohepatic *Helicobacter* species as a potential causative factor in inflammatory bowel disease: a meta-analysis. Medicine (Baltimore) 94:e1773. https://doi.org/10.1097/MD.0000000000001773
24. Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, Paster BJ et al (1998) Hepatic *Helicobacter* species identified in bile and

gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 114:755–763. https://doi.org/10.1016/s0016-5085(98)70589-x
25. Murata H, Tsuji S, Tsujii M, Fu HY, Tanimura H, Tsujimoto M et al (2004) *Helicobacter bilis* infection in biliary tract cancer. Aliment Pharmacol Ther 20(Suppl 1):90–94. https://doi.org/10.1111/j.1365-2036.2004.01972.x
26. Matsukura N, Yokomuro S, Yamada S, Tajiri T, Sundo T, Hadama T et al (2002) Association between *Helicobacter bilis* in bile and biliary tract malignancies: *H. bilis* in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract. Jpn J Cancer Res Gann 93:842–7. https://doi.org/10.1111/j.1349-7006.2002.tb01327.x
27. Fukuda K, Kuroki T, Tajima Y, Tsuneoka N, Kitajima T, Matsuzaki S et al (2002) Comparative analysis of *Helicobacter* DNAs and biliary pathology in patients with and without hepatobiliary cancer. Carcinogenesis 23:1927–1931. https://doi.org/10.1093/carcin/23.11.1927
28. Segura-López FK, Avilés-Jiménez F, Güitrón-Cantú A, Valdéz-Salazar HA, León-Carballo S, Guerrero-Pérez L et al (2015) Infection with *Helicobacter bilis* but not *Helicobacter hepaticus* was Associated with extrahepatic cholangiocarcinoma. Helicobacter 20:223–230. https://doi.org/10.1111/hel.12195
29. Tolia V, Nilsson H-O, Boyer K, Wuerth A, Al-Soud WA, Rabah R et al (2004) Detection of *Helicobacter ganmani*-like 16S rDNA in pediatric liver tissue. Helicobacter 9:460–468. https://doi.org/10.1111/j.1083-4389.2004.00266.x
30. Peng W, Li H, Xu Y, Yan L, Tang Z, Hossein Mohseni A et al (2021) Association of *Helicobacter bilis* Infection with the development of colorectal cancer. Nutr Cancer 73:2785–2795. https://doi.org/10.1080/01635581.2020.1862253
31. Steinbrueckner B, Haerter G, Pelz K, Weiner S, Rump J-A, Deissler W et al (1997) Isolation of *Helicobacter pullorum* from patients with enteritis. Scand J Infect Dis 29:315–318
32. Fresia P, Jara R, Sierra R, Ferrés I, Greif G, Iraola G et al (2017) Genomic and clinical evidence uncovers the enterohepatic species *Helicobacter valdiviensis* as a potential human intestinal pathogen. Helicobacter 22. https://doi.org/10.1111/hel.12425
33. Ceelen L, Decostere A, Verschraegen G, Ducatelle R, Haesebrouck F (2005) Prevalence of *Helicobacter pullorum* among patients with gastrointestinal disease and clinically healthy persons. J Clin Microbiol 43:2984–2986. https://doi.org/10.1128/JCM.43.6.2984-2986.2005
34. Stanley J, Linton D, Burnens AP, Dewhirst FE, Owen RJ, Porter A et al (1993) *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. J Gen Microbiol 139:2495–504. https://doi.org/10.1099/00221287-139-10-2495
35. van Wezel EM, van der Beek ESJ, Siebrecht MAN, Stel AJ, Wouthuyzen-Bakker M, Meessen NEL (2022) Pet-related bacterial zoonotic infections: three cases of severe infections in the immunocompromised host. IDCases 30:e01623. https://doi.org/10.1016/j.idcr.2022.e01623
36. Lardinois B, Belkhir L, Verroken A (2021) *Helicobacter canis*: a review of Microbiological and Clinical Features. Front Microbiol 12:814944. https://doi.org/10.3389/fmicb.2021.814944
37. Wang H, Gu Y, Zhou G, Chen X, Zhang X, Shao Z et al (2023) Helicobacter zhangjianzhongii sp. nov., isolated from dog feces. Front Genet 14:1240581. https://doi.org/10.3389/fgene.2023.1240581
38. Spergser J, Hess C, Loncaric I, Ramírez AS (2019) Matrix-assisted laser desorption ionization-time of flight mass spectrometry is a superior diagnostic tool for the identification and differentiation of mycoplasmas isolated from animals. J Clin Microbiol 57:e00316-e319. https://doi.org/10.1128/JCM.00316-19
39. Wingett SW, Andrews S (2018) FastQ Screen: A tool for multi-genome mapping and quality control. F1000Research 7:1338. https://doi.org/10.12688/f1000research.15931.2
40. Joshi N, Fass J (2011) Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33)[Software]. https://github.com/najoshi/sickle. Accessed 24 July 2014
41. Souvorov A, Agarwala R, Lipman DJ (2018) SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 4(19):153. https://doi.org/10.1186/s13059-018-1540-z
42. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1(25):3389–3402. https://doi.org/10.1093/nar/25.17.3389
43. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340
44. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 25(38):3022–3027. https://doi.org/10.1093/molbev/msab120
45. Letunic I, Bork P (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 44:W242-245. https://doi.org/10.1093/nar/gkw290
46. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 30(9):5114. https://doi.org/10.1038/s41467-018-07641-9
47. Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 16(10):2182. https://doi.org/10.1038/s41467-019-10210-3
48. Stoffel MH, Friess AE, Burnens A, Schmassmann A, Neiger R (2000) Distinction of gastric *Helicobacter spp*. in humans and domestic pets by scanning electron microscopy. Helicobacter 5:232–9. https://doi.org/10.1046/j.1523-5378.2000.00036.x
49. Kawamura Y, Tomida J, Morita Y, Fujii S, Okamoto T, Akaike T (2014) Clinical and bacteriological characteristics of *Helicobacter cinaedi* infection. J Infect Chemother Off J Jpn Soc Chemother 20:517–526. https://doi.org/10.1016/j.jiac.2014.06.007
50. On SL, Holmes B, Sackin MJ (1996) A probability matrix for the identification of campylobacters, helicobacters and allied taxa. J Appl Bacteriol 81:425–432. https://doi.org/10.1111/j.1365-2672.1996.tb03529.x
51. Vandamme P, Harrington CS, Jalava K, On SL (2000) Misidentifying helicobacters: the *Helicobacter cinaedi* example. J Clin Microbiol 38:2261–2266. https://doi.org/10.1128/JCM.38.6.2261-2266.2000
52. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM et al (2009) Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis Off Publ Infect Dis Soc Am 15(49):543–551. https://doi.org/10.1086/600885
53. Berlamont H, De Witte C, De Bruyckere S, Fox JG, Backert S, Smet A et al (2021) Differentiation of gastric *Helicobacter* species using MALDI-TOF mass spectrometry. Pathog Basel Switz 18(10):366. https://doi.org/10.3390/pathogens10030366