Yersinia canariae sp. nov., isolated from a human yersiniosis case

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ABSTRACT

A Gram-negative rod from the *Yersinia* genus was isolated from a clinical case of yersiniosis in the United Kingdom. Long read sequencing data from an Oxford Nanopore Technology (ONT) MinION in conjunction with Illumina HiSeq reads were used to generate a finished quality genome of this strain. Overall Genome Related Index (OGRI) of the strain was used to determine that it was a novel species within *Yersinia*, despite biochemical similarities to *Yersinia enterocolitica.* The 16S ribosomal RNA gene accessions are MN434982-MN434987 and the accession number for the complete and closed chromosome is CP043727. The type strain is CFS3336^T (=NCTC 14382^T/ =LMG *Accession under process*).

INTRODUCTION AND BACKGROUND

The majority of species within the *Yersinia* genus are considered to be non-pathogenic and are found broadly within the environment. Pathogenic members of *Yersinia* have been shown to evolve independently following the acquisition of virulence genes in select lineages (1). *Yersinia pestis*, arguably one of the most historically serious zoonotic pathogens reported (2), is the agent of bubonic, pneumonic, and septicaemic plague (3). Two other species, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, are the aetiological agents of the human gastrointestinal infection yersiniosis (4). As detailed in a 2016 report by the European Food Safety Authority, yersiniosis is the third most commonly reported zoonotic pathogen in Europe (5). *Yersinia* lends its namesake to the *Yersiniaceae* family within the *Enterobacteriales* order (6) and is comprised of 19 species, including the aforementioned pathogenic species and *Y. aldovae*, *Y. aleksiciae*, *Y. bercovieri*, *Y. entomophaga*, *Y. frederiksenii*, *Y. hibernica*, *Y. intermedia*, *Y. kristensenii*, *Y. massiliensis*, *Y. mollaretii*, *Y. nurmii*, *Y. pekkanenii*, *Y. rohdei*, *Y. ruckeri*, *Y. similis*, and *Y. wautersii*. Two additional subspecies are described with *Y. enterocolitica* subsp. *palearctica* and the recently characterised *Y. kristensenii* subsp. *rochesterensis* (7).

At a local NHS frontline hospital laboratory, diarrhoeic stool samples were tested with the GI PCR screening test (Fast-Track Diagnostics Bacterial gastroenteritis panel FTD-14.1-64 supplied by Launch Diagnostics). Specimens which tested positive for

Yersinia by PCR were then cultured on Cefsulodin irgasan (triclosan) novobiocin (CIN) agar at 28° C for 48 hours. Isolates were referred to the Gastrointestinal Bacteria Reference Unit (GBRU) in Public Health England (PHE) for further speciation and characterisation. As per statutory reporting requirements for infectious diseases, the laboratory reported confirmed cases (PCR and/or culture positive) to PHE's Second Generation Surveillance System (SGSS).

One such suspected *Yersinia* isolate, denoted NCTC 14382^T, was isolated from an adult human female in the United Kingdom after travel to the Canary Islands in 2018 (8). Identification of *Yersinia* to the species level by traditional biochemical methods is difficult due to heterogeneous biochemical phenotypes (9), thus all *Yersinia* isolates receipted at the GBRU are routinely sequenced via an Illumina HiSeq 2500 and subsequent bioinformatics speciation is based on a k-mer (18-mer) based approach comparing k-mers to a known reference database (8). The closest match for this isolate by number of k-mers in the database was *Yersinia enterocolitica.* Whole genome sequencing data characterised this isolate as ST333, utilising the multi-locus sequence type (MLST) scheme developed by Hall *et al*. (8,10). A phylogenetic tree previously revealed that the isolate did not cluster with *Y. enterocolitica* instead being located on a distinct branch (8). This study aims to resolve the taxonomic placement of this isolate as a new species within *Yersinia* with the support of genomic and biochemical data. As NCTC 14382^T was associated with travel to the Canary Islands, the name *Yersinia canariae* sp. nov. is proposed.

BIOCHEMICAL TESTS

API 20E strips were used in determining the phenotypes of *Y. canariae* NCTC 14382^T and two closely related *Yersinia* species through a gallery of biochemical tests. The latter is suitable for the identification of *Yersinia* species (11) but is dependent on incubation temperatures (12,13). Biological triplicates of the test *Yersinia* strains were assayed with API 20E test strips according to manufacturer's instructions and incubated at both 28° and 37° C for 24 hours (**Table 1**). Incubation of NCTC 14382^T at 28° C revealed this strain was capable of fermenting almost all carbon sources, similar to *Y. enterocolitica* 8081 (**Fig. 2**). *Y. canariae* NCTC 14382^T was negative for utilisation of ODC at 28° C in contrast to *Y. enterocolitica*. Inoculated API 20E strips incubated at 37° C produced more contrasting phenotypes notably with the loss of inositol fermentation for NCTC 14382^T when compared to *Y. enterocolitica* (**Fig. 3**). *Y. canariae* NCTC 14382^T was positive for ONPG utilisation in contrast to the other two strains tested at 37° C.

GENOME FEATURES

Y. canariae NCTC 14382^T was previously sequenced by an Illumina HiSeq 2500 at Public Health England using the Nextera XP library preparation kit following a retrospective study on yersiniosis isolates cultured from patients between April 2004 and March 2018 (8). To generate a finished quality genome, NCTC 14382^T was grown in Luria-Bertani (LB) broth (Sigma) for 18 h at 25° C. Genomic DNA was extracted with a Wizard Genomic DNA Purification Kit (Promega) following the recommended manufacturer's protocol. DNA was sequenced on the ONT MinION R9.4 flowcell (FLO-MIN106) for approximately 16 hours.

For ONT MinION data, the run metrics were inspected using NanoPlot (version 1.0) (14) before raw FAST5 files were base-called using Guppy (version 3.2.2) with the high accuracy model to FASTQ files. Adapters were trimmed from the raw reads by Porechop (version 0.2.4) using default parameters for SQK-RAD004 before the genome was *de*

novo assembled with Flye (version 2.5) (15,16). The best assembly parameters were empirically determined to include the option flags "meta" and "plasmid" with coverage reduced to 30X for initial contig assembly based on a predicted genome size of ~4.73 Mbp as informed by *de novo* assembly of short read Illumina data (17). This produced a single contiguous chromosome for which the final consensus sequence was determined following four iterative rounds of long read polishing with Racon (version 1.4.3) (18) using the high accuracy base-called reads produced by Guppy that were previously adaptor trimmed by Porechop. A final round of consensus sequence correction was performed with the same long read data using Medaka (version 0.8.2). Lastly, short read Illumina data were aligned using minimap2 (version 2.17) (19) producing BAM files that were sorted and indexed with Samtools (version 1.9) (20) before four iterative rounds of short read polishing with Pilon (version 1.23) (21).

After assembly, a circular finished quality chromosome of 4,7101,54 bp devoid of any plasmid was generated. Annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) identified 4,370 genes of which 4,132 were coding. 8 copies of the 5S rRNA genes, 7 copies of the 16S rRNA genes, 7 copies of the 23S rRNA genes, 81 tRNA genes, and 6 non-coding RNA genes were present, resulting in a total of 109 RNA genes.

As the genome was corrected by multiple rounds with long read aware polishing, the most common 16S rRNA gene allele (Accession: MN434982) was extracted from the genome for analysis. The full length 16S rRNA gene was queried in the EzBioCloud 16S database as previously recommended by Chun *et al.* (**Table 2**) (22,23). For much of the type strains of *Yersinia* species, NCTC 14382^T showed at least 98.7% or higher 16S rRNA gene similarity and thus was queried for overall genomic related index (OGRI).

The average nucleotide identity (ANI) of NCTC 14382^T was determined by FastANI (v1.2) against the type sequences of all other *Yersinia* species (24). *Y. canariae* NCTC 14382^T was most closely related to *Y. hibernica*, *Y. enterocolitica* subsp. *enterocolitica*, *Y. enterocolitica* subsp. *palearctica*, *Y. kristensenii* subsp. *rochesterensis*, and *Y. kristensenii* subsp. *kristensenii* based on ANI values (**Table 3**). However, the ANI values for NCTC 14382^T are below the threshold of ≤95% ANI when compared to the type strains of other *Yersinia* species, thus suggesting taxonomic placement of NCTC 14382^T into a novel species. The digital DNA-DNA hybridization (dDDH) values were calculated with the Type (Strain) Genome Server hosted by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (25). Using the recommended formula d_4 (25,26) with the BLAST+ local alignment tool, *in silico* DNA-DNA hybridization values of NCTC 14382^T revealed additional OGRI data for the support of a novel species (**Table 3**).

A phylogenetic tree based on conserved core sequences used in a whole genome alignment was generated by Parsnp as previously described (27). The resulting phylogenetic tree was visualized in EvolView2 (28) and showed that *Y. canariae* clustered distinctly from other *Yersinia* species and was most closely related to the newly described *Y. hibernica* (27) (**Fig. 1**).

On the basis of the biochemical profile, phylogenetic relationships, and OGRI data of isolate NCTC 14382^T, evidence for a new species within *Yersinia* is conclusive in which the name *Yersinia canariae* sp. nov. is proposed.

Description of *Yersinia canariae* sp. nov.

Yersinia canariae (ca.na'ri.ae. N.L. gen. n. *canariae* from the Canary Islands in which this strain was associated with travel to the Canary Islands, *Canariae insulae*).

Cells grow aerobically at 25-37 °C on LB agar, producing 1.5-2.0 mm diameter colonies after 24 hours. At 28 °C, cells are positive for ortho-nitrophenyl-β-D-galactopyranoside hydrolysis, urease utilisation, indole production and fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, D-sucrose, amygdalin, and L-arabinose. At 28 °C, cells are negative for utilisation of L-arginine, L-lysine, L-ornithine, trisodium citrate, H_2S production, tryptophan deaminase, gelatinase, L-rhamnose fermentation, and D-melibiose fermentation. In contrast to growth at 28 °C, cells do not ferment inositol at 37 °C. The DNA G+C content is 47.2% and the chromosomal length of the type strain is 4710154 bp.

The type strain, CFS3336^T (=NCTC 14382^T =LMG *Accession under process*), was isolated in the United Kingdom from a yersiniosis case associated with travel to the Canary Islands. The complete genome of NCTC 14382^T has been deposited into GenBank (accession number, CP043727).

Table 1

	28 °C			37 °C		
	1	2	3	1	2	3
ONPG	+	+	-	V	+	-
ADH	-	-	-	-	-	-
LDC	-	-	-	-	-	-
ODC	+	-	-	+	-	-
CIT	-	-	-	-	-	-
H2S	-	-	-	-	-	-
URE	+	+	+	+	+	+
TDA	-	-	-	-	-	-
IND	+	+	-	+	+	-
VP	-	-	-	-	-	-
GEL	-	-	-	-	-	-
GLU	+	+	+	+	+	+
MAN	+	+	+	+	+	+
INO	+	+	+	+	-	-
SOR	+	+	+	+	+	+
RHA	-	-	-	-	-	-
SAC	+	+	-	+	+	-
MEL	-	-	-	-	-	-
AMY	+	+	+	+	+	-
ARA	+	+	+	+	+	+

API 20E results for *Y. enterocolitica* 8081 (1), *Y. canariae* NCTC 14382^{T} (2), and *Y. hibernica* CFS1934 (3) at 28 °C and 37 °C. + = positive, - = negative, V = variable.

Table 2

Name	Strain	Accession	Pairwise Similarity (%)	Mismatch /Total nt
Yersinia frederiksenii	ATCC 33641	JPPS01000006	99.11	13/1465
Yersinia pekkanenii	CIP 110230	CWJL01000114	98.91	16/1465
Yersinia hibernica	CFS1934	MK129259	98.91	16/1465
Yersinia kristensenii subsp. *rochesterensis*	EPLC-04	KJ606916	98.90	16/1457
Yersinia aldovae	ATCC 35236	AF366376	98.84	17/1461
Yersinia rohdei	ATCC 43380	ACCD01000072	98.77	18/1465
Yersinia enterocolitica subsp. *palearctica*	Y11	FR729477	98.77	18/1465
Yersinia massiliensis	CCUG 53443	CAKR01000050	98.77	18/1465
Yersinia nurmii	CIP 110231	CPYD01000031	98.77	18/1465
Yersinia intermedia	ATCC 29909	AF366380	98.77	18/1461
Yersinia entomophaga	MH96	CP010029	98.70	19/1464
Yersinia mollaretii	ATCC 43969	AF366382	98.70	19/1461
Yersinia kristensenii subsp. *kristensenii*	ATCC 33638	ACCA01000078	98.63	20/1465
Yersinia bercovieri	ATCC 43970	AF366377	98.56	21/1461
Yersinia pestis	NCTC 5923	AF366383	98.43	23/1461
Yersinia pseudotuberculosis	NBRC 105692	BAUR01000130	98.36	24/1465
Yersinia similis	Y228	CP007230	98.29	25/1465
Yersinia wautersii	12-219N1	HG326166	98.29	25/1464
Yersinia aleksiciae	DSM 14987	CP011975	98.16	27/1465
Yersinia enterocolitica subsp. *enterocolitica*	ATCC 9610	JPDV01000006	98.02	29/1465
Yersinia ruckeri	ATCC 29473	JPPT01000003	97.82	32/1465

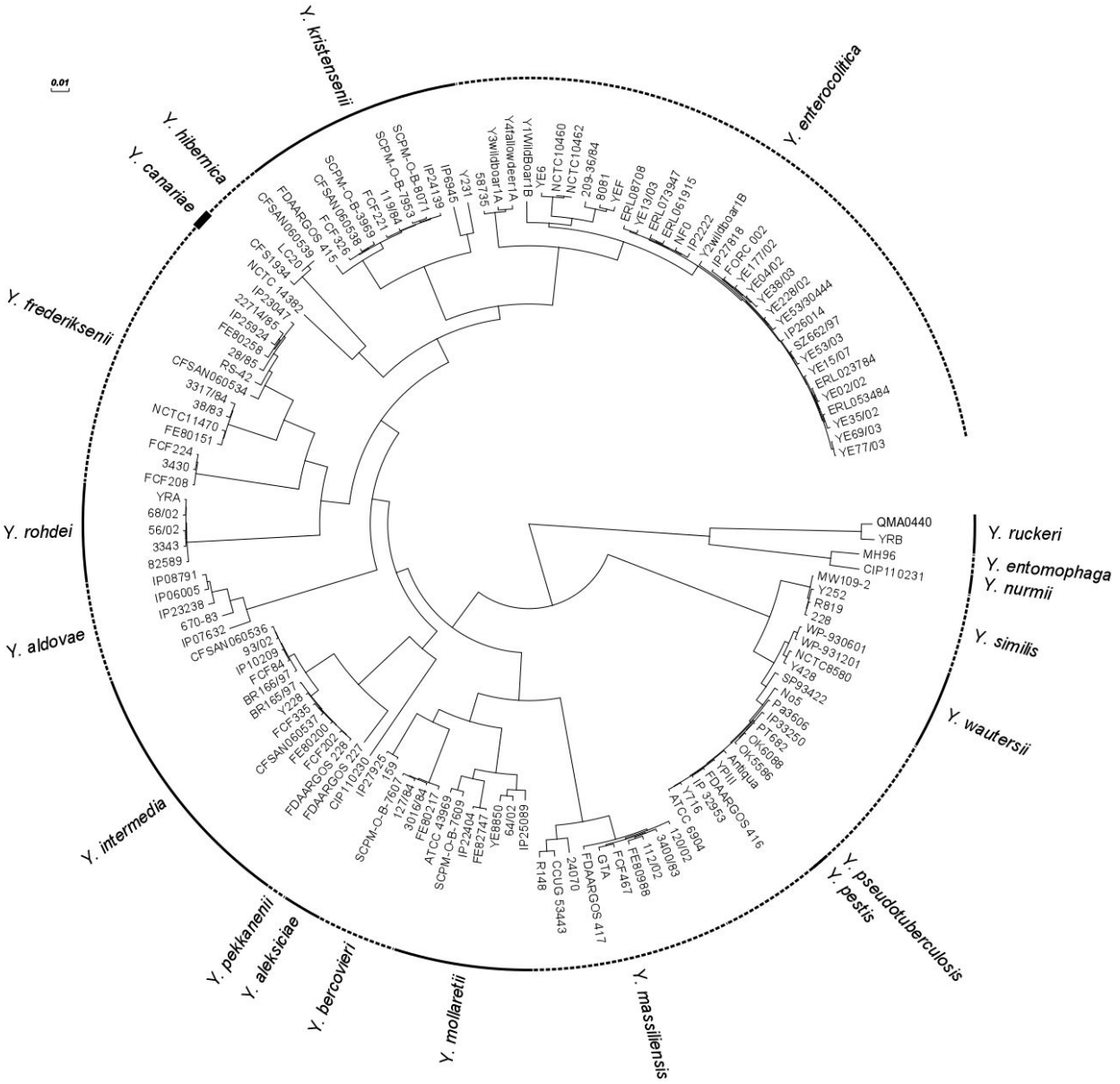
16S rRNA gene pairwise similarity of *Y. canariae* (Accession: MN434982) to other type strains of *Yersinia*. Nucleotide mismatch is based on differences between the *Y. canariae* 16S rRNA gene to reference sequences.

Table 3

	ANI	DDH	Model C.I.
Yersinia hibernica	90.10	39.2	[36.7 – 41.7]
Yersinia enterocolitica subsp. enterocolitica	87.79	33.3	[30.9 – 35.8]
Yersinia enterocolitica subsp. palearctica	87.72	33.2	[30.8 – 35.8]
Yersinia kristensenii subsp. rochesterensis	87.69	33.0	[30.6 – 35.5]
Yersinia kristensenii subsp. kristensenii	87.54	32.8	[30.4 – 35.3]
Yersinia frederiksenii	85.61	28.3	[26.0 – 30.8]
Yersinia rohdei	85.29	28.1	[25.7 – 30.6]
Yersinia aleksiciae	84.92	27.7	[25.4 – 30.2]
Yersinia pekkanenii	84.65	27.6	[25.2 – 30.1]
Yersinia bercovieri	84.64	27.0	[24.7 – 29.5]
Yersinia mollaretii	84.60	27.1	[24.7 – 29.5]
Yersinia aldovae	84.56	26.7	[24.4 – 29.2]
Yersinia intermedia	84.54	27.0	[24.6 – 29.4]
Yersinia massiliensis	83.42	25.3	[22.9 – 27.7]
Yersinia pestis	82.68	25.9	[23.5 – 28.3]
Yersinia pseudotuberculosis	82.58	25.7	[23.4 – 28.2]
Yersinia similis	82.57	25.8	[23.5 – 28.3]
Yersinia wautersii	82.43	25.4	[23.1 – 27.9]
Yersinia entomophaga	81.19	22.2	[19.9 – 24.6]
Yersinia ruckeri	81.12	21.9	[19.6 – 24.3]
Yersinia nurmii	80.93	21.8	[19.5 – 24.2]

ANI and DDH (d_4 formula) of *Y. canariae* NCTC 14382^T queried against type strains of other *Yersinia* species. Closely related *Y. hibernica* was at 90.10% ANI and 39.2% DDH, well under the 95% ANI and 70% DDH cutoff proposed by Chun *et al.* (22).

Figure 1



Taxonomic placement of *Y. canariae* NCTC 14382^{T} in relation to other *Yersinia* species on the basis of whole genome alignment of conserved core sequences.

Figure 2
API 20E at 28 °C

Growth of *Y. enterocolitica* 8081 (**Ye**), *Y. canariae* NCTC 14382^{T} (**Yc**), and *Y. hibernica* CFS1934 (**Yh**) at 28 °C after 20 hours. Boxes highlight biochemical features distinct from *Y. enterocolitica* (red) or *Y. hibernica* (blue).

Figure 3
API 20E at 37 °C.

Growth of *Y. enterocolitica* 8081 (**Ye**), *Y. canariae* NCTC 14382^{T} (**Yc**), and *Y. hibernica* CFS1934 (**Yh**) at 37 °C after 20 hours. Boxes highlight biochemical features distinct from *Y. enterocolitica* (red) or *Y. hibernica* (blue) or both (black).

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