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First isolation of *Yersinia entomophaga* in human urinary tract

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Abstract

Yersinia entomophaga is an insect pathogen first isolated from larvae of *Coleoptera* in New Zealand in 2011. We report here the first isolation of *Y. entomophaga* from human urine. Using whole-genome sequencing, we confirmed the presence of specific chromosomal virulence genes and identified a plasmid harbouring a quinolone resistance gene.

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Introduction

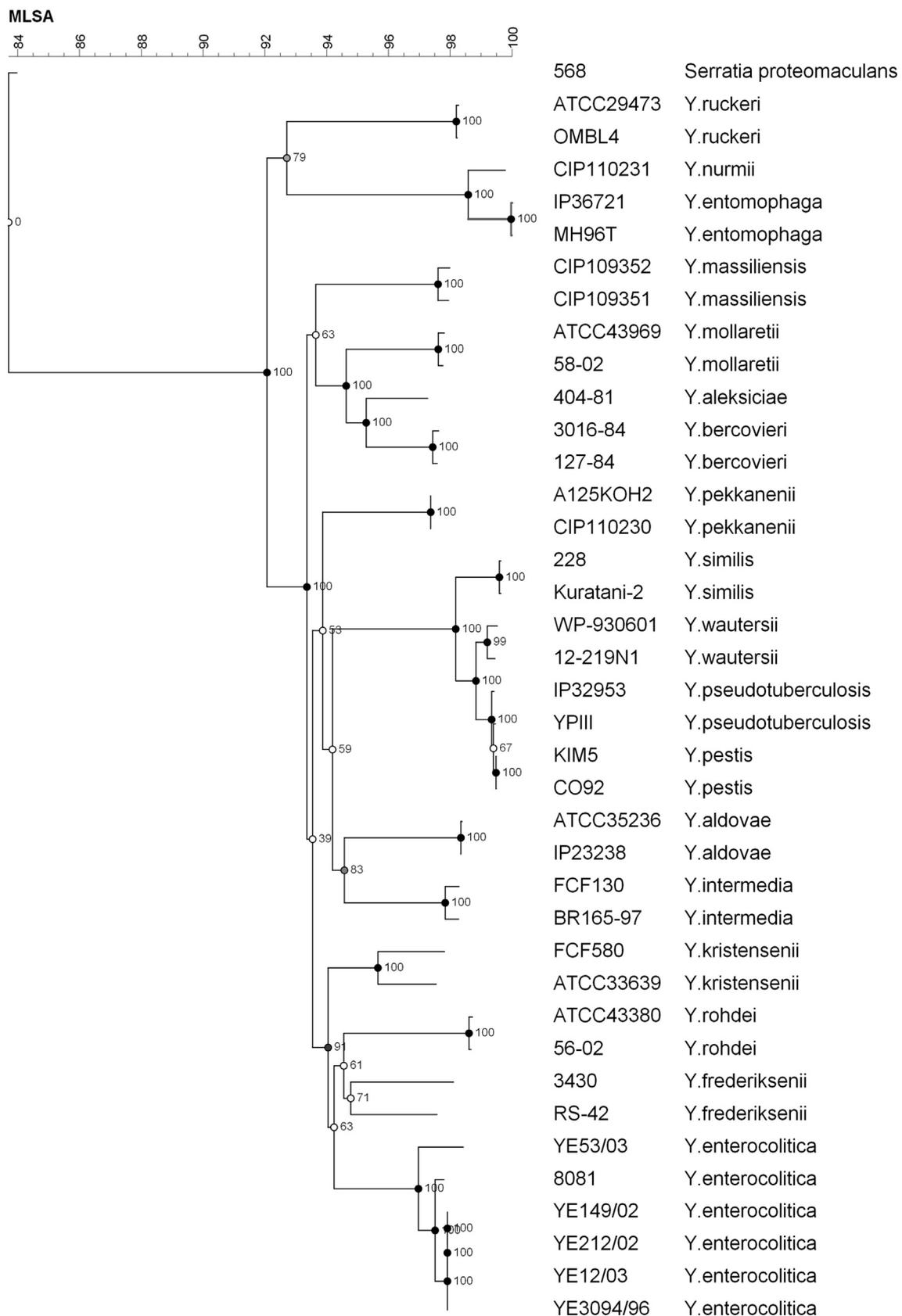
Within the *Yersinia* genus, three species are human pathogens: *Y. pestis*, which is the causative agent of plague, and two enteropathogens, *Y. pseudotuberculosis* and *Y. enterocolitica*, transmitted by ingestion of contaminated food. These enteropathogens are usually isolated in stools, and less frequently in lymph nodes and blood. *Yersinia entomophaga*, a novel species described in 2011 in New Zealand, is an insect pathogen which kills a wide range of coleopteran, lepidopteran and orthopteran species [1] but has never been isolated in humans.

We report here the first isolation of *Y. entomophaga* from human urine in a case of catheter-associated asymptomatic bacteriuria (CA-ASB).

Case report

The patient was an 85-year-old retired man. He did not travel abroad, usually stayed at home and practiced fishing. His medical history was remarkable for obesity, diabetes, atrial fibrillation, high blood pressure, coronary disease, chronic obstructive pulmonary disease and thyroid insufficiency. He was hospitalized in January 2015 in Barbezieu Hospital (France) for heart and respiratory decompensation with hypertension, and developed acute urinary retention, leading to long-term urinary catheterization with a 100% silicone and latex-free transurethral Foley catheter (178305; Teleflex). All attempts at removing the catheter resulted in urinary retention, so a laser photovaporization of the prostate was scheduled at the urology department of Angoulême Hospital (France) in April 2015. Meanwhile, the patient stayed at home. He had no special dietary regimen and no contact with people coming back from New Zealand; nor did he work with biological insecticides.

According to the presurgical instructions, a urine sample was collected 2 days before, the catheter was replaced the day before, and a second-generation cephalosporin course was provided during surgery. The urine microscopic examination showed 60 000 leucocytes/mL and 326 000 erythrocytes/mL, and the urine



culture indicated the presence of $>10^6$ CFU/mL *Enterococcus faecalis* as well as $>10^6$ CFU/mL *Yersinia* spp. The species assignment of the *Yersinia* strain proposed by matrix-assisted desorption ionization–time of flight mass spectrometry (Bruker Daltonics) was *Y. pseudotuberculosis* or *Y. enterocolitica*. The score values were 1.925 and 1.821 respectively, allowing only a probable identification of the genus without species consistency. Thus, the strain was sent for characterization to the French *Yersinia* National Reference Laboratory (YNRL).

Antibiotic susceptibility was tested using a broth dilution method using the BD Phoenix Automated Microbiology System (Becton Dickinson). The *E. faecalis* strain was susceptible to ampicillin, imipenem, nitrofurantoin, moxifloxacin, teicoplanin, vancomycin, linezolid and chloramphenicol, intermediate to trimethoprim/sulfamethoxazole and resistant to aminoglycosides and macrolides. The *Yersinia* spp. strain was susceptible to third-generation cephalosporins, quinolones, fluoroquinolones, carbapenems, trimethoprim/sulfamethoxazole, fosfomycin, nitrofurantoin, aminoglycosides and association ticarcillin/clavulanate; and resistant to ampicillin, ticarcillin, and first-generation cephalosporins, as usually observed for *Y. enterocolitica* species.

Characterization of *Yersinia* strain

YNRL confirmed the *Yersinia* genus and assigned the *Y. entomophaga* species by metabolic tests: API20E and API50CH strips (bioMérieux), pyrazinamidase and lipase activities. The key differential characteristics were rhamnose negative, sucrose positive, melibiose positive, L-arabinose negative, D-xylose negative, urease negative, pyrazinamidase positive and lipase positive. The strain belonged to the O:7,8-8-8,19 serotype and was recorded as IP36721 in the YNRL strain collection.

The whole genome of the strain was sequenced with the Nextera XT protocol using a NextSeq 500 sequencer (Illumina). A *de novo* assembly of the genomes was performed as described by Saraka et al. [2]. The whole genome shotgun project of the IP36721 strain was deposited at the DNA Data Bank of Japan, European Nucleotide Archive and GenBank under accession number QCZL00000000. Multilocus sequence analysis based on *glnA*, *recA*, *gyrB* and *hsp60* housekeeping genes was performed [3]. The four concatenated sequences were compared to the sequences of a set of reference strains belonging to all *Yersinia* species [4,5] (Fig. 1). The strain branched to the MH96T *Y. entomophaga* type strain, confirming the species assignment.

The presence of virulence factors, usually found in the *Yersinia* strains pathogenic for humans, was investigated using Basic Local Alignment Search Tool (BLAST) of reference sequences of virulence genes on the IP36721 strain genome. The main chromosomal and plasmid virulence genes were not found in IP36721 strain (Table 1). The *blaA* and *blaB* genes encoding a constitutive class A penicillinase and an inducible class C cephalosporinase respectively in *Y. enterocolitica* were both present, but with low sequence similarities, 67% and 66% identity for *blaA* and *blaB* respectively and a very low coverage (42%) for *blaA*.

The presence of the main virulence factors described in *Y. entomophaga* MH96T species was investigated using BLAST on the genome of the IP36721 strain [6]. The genes located on the pathogenicity island encoding the insecticidal toxin complex in the species *Y. entomophaga* were all present in IP36721 (100% coverage and 99% identity) (Table 1). Amino acid sequences alignment showed 100% identity for YenA1, YenA2 and YenC2 and one synonymous mutation for YenC1, YenB, Chi1 and Chi2.

The comparison of the MH96T and IP36721 genomes showed 5071 single nucleotide polymorphisms regularly scattered along the genome, representing 1.1% of genome divergence and thus showing a high diversity between the two strains. Additional nucleotides present only in the IP36721 strain allowed the identification of a contig identical to the pM510 plasmid from *Proteus mirabilis* carrying the *qnrD* gene conferring a low-level quinolone resistance.

Discussion

This reported case is considered a CA-ASB, as defined by the presence of $\geq 10^5$ CFU/mL of one or more bacterial species in a single catheter urine specimen in a patient without symptoms of urinary tract infection [7]. In CA-ASB, bacteria usually originate from the periurethral area and form a biofilm along the catheter surface. Bacteria grow in the biofilm and travel up to the bladder, colonize the urinary bladder lining and are released in the urine flow [8]. *E. faecalis* is isolated in up to 30% of catheter-associated urinary tract infections. It is a normal inhabitant of the intestinal tract and also a well-known opportunistic pathogen. Its ability to overcome the body-mediated inflammation caused by the catheterization and to form a biofilm on the catheter promotes its growth in the urinary tract [9,10]. However, isolation of *Yersinia* strains in urine is rarely reported [11–13] even though *Yersinia*

FIG. 1. Phylogenetic tree comparing *Yersinia entomophaga* IP36721 isolate to publicly available reference strains [4,5]. Neighbour-joining tree was constructed from concatenated sequences of *glnA*, *recA*, *gyrB* and *hsp60* genes (≈ 2000 bp) compared using method based on Jukes-Cantor distance matrix [3]. Bootstrap values obtained after 1000 replicates are given at nodes. Tree was rooted using *Serratia proteomaculans*.

TABLE 1. Detection of virulence-associated genes in IP36721 *Yersinia entomophaga* strain

| Origin | Gene | Product/function | IP36721 strain |
|--------------------------------------|-----------------------------|---|----------------|
| <i>Yersinia</i> pathogenic for human | <i>inv</i> ^a | Invasin/attachment and entry into host cells | – |
| | <i>aif</i> ^a | Ail/attachment, invasion and resistance to complement-mediated lysis | – |
| | <i>myfA</i> ^a | Mucoid <i>Yersinia</i> factor/fimbrial antigen and putative adhesin | – |
| | <i>irp2</i> ^{a,b} | HMWP2/ferric yersiniabactin uptake | – |
| | <i>fyuA</i> ^{a,b} | Yersiniabactin receptor FyuA/ferric yersiniabactin uptake | – |
| | <i>yadA</i> ^c | <i>Yersinia</i> adhesin A/adhesion to host ileo-caecal epithelium | – |
| | <i>yopB</i> ^c | <i>Yersinia</i> outer protein (Yop) B/translocon of the type III secretion system | – |
| | <i>yopD</i> ^c | YopD/translocon of the type III secretion system | – |
| | <i>yopT</i> ^c | YopT/effector protein | – |
| | <i>ycsF</i> ^c | YcsF/needle of the type III secretion system | – |
| | <i>lcrV</i> ^c | LcrV/needle tip of the type III secretion system | – |
| MH96T <i>Y. entomophaga</i> | <i>yenA1</i> ^{a,b} | YenA1/insecticidal toxin complex protein | + |
| | <i>yenA2</i> ^{a,b} | YenA2/insecticidal toxin complex protein | + |
| | <i>yenB</i> ^{a,b} | YenB/insecticidal toxin complex protein | + |
| | <i>yenC1</i> ^{a,b} | YenC1/insecticidal toxin complex protein | + |
| | <i>yenC2</i> ^{a,b} | YenC2/insecticidal toxin complex protein | + |
| | <i>chi1</i> ^{a,b} | Chi1/insecticidal toxin complex chitinase | + |
| | <i>chi2</i> ^{a,b} | Chi2/insecticidal toxin complex chitinase | + |

^aLocated on chromosome.
^bLocated on high-pathogenicity island.
^cLocated on plasmid.

spp. may transiently colonize the intestinal flora and therefore could be present in the periurethral area and introduced in the urethra. However, in the present case, we ignored how the patient was infected, and the presence of *Y. entomophaga* in stools and blood was not investigated.

Y. entomophaga has never been isolated in humans. Because this species was only described in 2011, automated systems of bacterial identification have not yet included the specific pattern of characteristics of *Y. entomophaga* for its distinction from other *Yersinia* species. Mass spectrometry also fails to identify microorganisms that are not included in the reference library. However, if the taxonomic assignation is not validated by the system of identification, then the strain is sent to a reference laboratory for characterization.

Presurgical instructions require laboratories assess many preoperative samples a day. These samples are mostly collected from asymptomatic patients, and the results are often normal. However, these preoperative instructions may sometimes result in the isolation of an uncommon species, such as *Y. entomophaga*.

E. faecalis has been shown to promote innate immune suppression and polymicrobial catheter-associated urinary tract

infection [14]. Thus, we can hypothesize that *E. faecalis* favoured the formation of a biofilm and promoted the growth of *Y. entomophaga* in urine. *Y. entomophaga* is considered nonpathogenic in humans because it does not possess the virulence factors usually associated with *Yersinia* pathogenicity, but it has been shown to kill the larvae of a wide range of insects. The main virulence determinant is a toxin complex that causes loss of gut epithelial integrity, allowing the bacterium to enter the insect haemocoelic cavity. A recent analysis of the draft genome sequence of MH96T strain showed that it encodes an array of toxins, including two type III secretion systems, five *rhs*-associated gene clusters and distant orthologs of some mammalian toxins [15]. We cannot rule out the notion that these virulence factors play a role in the pathogenesis of a human *Y. entomophaga* infection. In addition, the IP36721 *Y. entomophaga* strain has acquired a plasmid carrying a *qnrD* gene, which encodes quinolone resistance. This suggests that *Y. entomophaga* is able to acquire virulence factors when in contact with other pathogens and thus could be more pathogenic to humans than previously appreciated. Even though the pathogenicity of *Y. entomophaga* for humans still remains unclear, we report here its first isolation in Europe and its ability to multiply in humans.

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Conflict of interest

None declared.

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