First isolation of Yersinia entomophaga in human urinary tract
A.-S. Le Guern, Cyril Savin, S. Bremont, G. Payro, D. Bon, E. Carniel, Javier Pizarro-Cerdá

To cite this version:
A.-S. Le Guern, Cyril Savin, S. Bremont, G. Payro, D. Bon, et al.. First isolation of Yersinia entomophaga in human urinary tract. New Microbes and New Infections, Wiley Online Library 2018, 26, pp.3-7. 10.1016/j.nmni.2018.08.002 . pasteur-02545824

HAL Id: pasteur-02545824
https://hal-pasteur.archives-ouvertes.fr/pasteur-02545824
Submitted on 17 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives| 4.0 International License
First isolation of *Yersinia entomophaga* in human urinary tract

A.-S. Le Guern¹, C. Savin¹, S. Brémont¹, G. Payro², D. Bon¹, E. Carniel¹ and J. Pizarro-Cerdá¹

¹) Yersinia National Reference Laboratory, Yersinia Research Unit and WHO Collaborating Center for the Yersiniae, Institut Pasteur, Paris, 2) Laboratoire Cerdibio Charentes, Saintes and 3) Department of Surgery-Urology, Centre Hospitalier Général, Angoulême, France

**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.

© 2018 The Author(s). Published by Elsevier Ltd.

**Keywords:** CA-ASB, catheter-associated asymptomatic bacteriuria, catheter-associated urinary tract infection, CAUTI, urinary tract infection, Yersinia, yersiniosis

**Original Submission:** 29 May 2018; **Revised Submission:** 23 July 2018; **Accepted:** 3 August 2018

**Article published online:** 11 August 2018

**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, nitrozofurantoin, moxifloxacin, teicoplanin, vancomycin, linezolid and chloramphenicol, intermediate to trimethoprim/sulfamethoxazole and resistant to aminoglycosides and macrolides. The Y. entomophaga 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 positive, urease negative, pyrazinamidase positive and lipase positive. The strain allowed the identification of the species assignation.

This reported case is considered a CA-ASB, as defined by the presence of >10^6 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

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. enteromophaga 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 YenCl, 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 pMS10 plasmid from Proteus mirabilis carrying the qnrD gene conferring a low-level quinolone resistance.

Discussion

This 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 assignation.

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 proteamaculans.

© 2018 The Author(s). Published by Elsevier Ltd. NMNI, 26, 3–7
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
TABLE 1. Detection of virulence-associated genes in IP36721
Yersinia entomophaga strain

| Origin       | Gene | Product/function                                      | IP36721 strain |
|--------------|------|-------------------------------------------------------|----------------|
| Yersinia pathogenic for human | ntv  | Invasin/attachment and entry into host cells          | −              |
|              | rfa  | Ail/attachment, invasion and resistance to complement-mediated lysis | −              |
|              | myrA | Muscid Yersinia factor/fimbrial antigen and putative adhesin | −              |
|              | yps2 | HMWP2/ferric yersiniabactin uptake                      | +              |
|              | fyuA | Yersiniabactin receptor FyuA/ferric yersiniabactin uptake | −              |
|              | yadA | Yersinia adhesin A/adhesion to host ileo-caecal epithelium | −              |
|              | yopD | Yersinia outer protein (Yop) B/ translocon of the type III secretion system | −              |
|              | yopD | YopD/translocon of the type III secretion system       | −              |
|              | yopE | YopE/effector protein                                  | −              |
|              | yopT | YopT/effector protein                                  | −              |
|              | lcrV | LcrV/needle tip of the type III secretion system       | −              |
| MH96T Y. entomophaga | yenA1 | YenA1/insecticidal toxin complex protein                | +              |
|              | yenA2 | YenA2/insecticidal toxin complex protein                | +              |
|              | yenB  | YenB/insecticidal toxin complex protein                 | +              |
|              | yenC1 | YenC1/insecticidal toxin complex protein                | +              |
|              | yenC2 | YenC2/insecticidal toxin complex protein                | +              |
|              | chi1  | Chi1/insecticidal toxin complex chitinase              | +              |
|              | chi2  | Chi2/insecticidal toxin complex chitinase              | +              |

*Located on chromosome.
*Located on high-pathogenicity island.
*Located 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.

Acknowledgements

Supported by the Institut Pasteur and Santé publique France. We thank P. Terrade for providing clinical information on the patient.

Conflict of interest

None declared.

References

[1] Hurst MRH, Becher SA, Young SD, Nelson TL, Glare TR. Yersinia entomophaga sp. nov., isolated from the New Zealand grass grub Casteljula zealandica. Int J Syst Evol Microbiol 2011;61:844–9.
[2] Saraka D, Savin C, Kouassi S, Cisse B, Koffi E, Cabanel N, et al. Yersinia enterocolitica, a neglected cause of human enteric infections in Côte d'Ivoire. PLoS Neglect Trop Dis 2017;11: e0005216.
[3] Kotetishvili M, Kreger A, Wauters G, Morris JG, Sulakvelidze A, Stine OC. Multilocus sequence typing for studying genetic relationships among Yersinia species. J Clin Microbiol 2005;43:2674–84.
[4] Reuter S, Connor TR, Barquist L, Walker D, Feltwell T, Harris SR, et al. Parallel independent evolution of pathogenicity within the genus Yersinia. Proc Natl Acad Sci U S A 2014;111:6768–73.
[5] Savin C, Martin L, Bouchier C, Filali S, Chenau J, Zhou Z, et al. The Yersinia pseudotuberculosis complex: characterization and delineation of a new species, Yersinia wautersii. Int J Med Microbiol 2014;304:452–63.

[6] Hurst MRH, Jones SA, Binglin T, Harper LA, Jackson TA, Glare TR. The main virulence determinant of Yersinia entomophaga MH96 is a broad-host-range toxin complex active against insects. J Bacteriol 2011;193:1966–80.

[7] Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 international clinical practice guidelines from the Infectious Diseases Society of America. Clin Infect Dis 2010;50:625–63.

[8] Nicolle LE. Catheter associated urinary tract infections. Antimicrob Resist Infect Control 2014;3:23.

[9] Guinon PS, Hanin TJ, Ford B, Caparon MG, Hultgren SJ. Enterococcus faecalis overcomes foreign body–mediated inflammation to establish urinary tract infections. Infect Immun 2013;81:329–39.

[10] Seno Y, Kariyama R, Mitsuhasha R, Monden K, Kumon H. Clinical implications of biofilm formation by Enterococcus faecalis in the urinary tract. Acta Med Okayama 2005;59:79–87.

[11] Lupi A, Poletti F, Mondino V, Canale C, Leonardo L, Rognoni A, et al. Subacute endocarditis caused by Yersinia enterocolitica: a case report. Scand J Infect Dis 2013;45:329–33.

[12] Naeli B, Raul R. Chronic prostatitis due to Yersinia pseudotuberculosis. J Clin Microbiol 1998;36:856.

[13] Crvchova V, Grodin C. Urinary infection due to Yersinia pseudotuberculosis. Vie Med Can Fr 1973;2:3–5.

[14] Tien BYQ, Goh HMS, Chong KKL, Bhaduri-Tagore S, Holec S, Dress R, et al. Enterococcus faecalis promotes innate immune suppression and polymicrobial catheter-associated urinary tract infection. Infect Immun 2017;85. e00378-17.

[15] Hurst MRH, Beattie A, Altermann E, Moraga RM, Harper LA, Calder J, et al. The draft genome sequence of the Yersinia entomophaga entomopathogenic type strain MH96T. Toxins (Basel) 2016;8:E143.