



HAL
open science

Inquilinus limosus and Cystic Fibrosis

Fadi Bittar, Anne Leydier, Emmanuelle Bosdure, Alexandre Toro, Martine Reynaud-Gaubert, Stéphanie Boniface, Nathalie Stremler, Jean-Christophe Dubus, Jacques Sarles, Didier Raoult, et al.

► **To cite this version:**

Fadi Bittar, Anne Leydier, Emmanuelle Bosdure, Alexandre Toro, Martine Reynaud-Gaubert, et al.. Inquilinus limosus and Cystic Fibrosis. *Emerging Infectious Diseases*, 2008, 14 (6), pp.993 - 995. 10.3201/eid1406.071355 . hal-01478698

HAL Id: hal-01478698

<https://amu.hal.science/hal-01478698>

Submitted on 13 Oct 2023

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 4.0 International License

- rural South African district hospitals: an epidemiological modelling study. *Lancet*. 2007;370:1500–7.
- Brewer T. Extensively drug-resistant tuberculosis and public health. *JAMA*. 2007;298:1861.
 - Kim HR, Hwang SS, Kim HJ, Lee SM, Yoo CG, Kim YW, et al. Impact of extensive drug resistance on treatment outcomes in non-HIV-infected patients with multidrug-resistant tuberculosis. *Clin Infect Dis*. 2007;45:1290–5.
 - Demographic and Health Survey, Lesotho 2004.
 - World Health Organization. Tuberculosis profile, 2007 [cited 2007 Feb 21]. Available from <http://www.who.int/tb/en>
 - National Tuberculosis and Leprosy Program. Kingdom of Lesotho annual report, 2007. Maseru (Lesotho): The Program; 2007
 - Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martin-Casabona N, et al. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis*. 2007;13:380–7.
 - Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet*. 2006;368:1575–80.

Address for correspondence: Jennifer Furin, Brigham and Women's Hospital, Division of Social Medicine and Health Inequalities, 651 Huntington Ave, 7th Floor, Boston, MA 02115, USA; email: jfurin@partners.org

Inquilinus limosus and Cystic Fibrosis

To the Editor: *Inquilinus limosus*, a new multidrug-resistant species, was reported in 1999 as an unidentified gram-negative bacterium in a lung transplant patient with cystic fibrosis (CF) (1). This species was later characterized by the description of 7 new isolates of *I. limosus* and 1 isolate of *Inquilinus* sp. (2). Infections and colonizations by *I. limosus* have been documented mainly in adolescent or adult patients with CF. To date, 8 clinical cases have been described in Germany

(3,4), 1 case in the United States (1), 5 cases in France (5), and 1 case in the United Kingdom (6) (Table). Only 1 isolate of *Inquilinus* sp. has been recovered from blood samples of a patient without CF who had prosthetic valve endocarditis (7).

Because this bacterium is not recorded in all commercial identification system databases currently available, a longitudinal study for *I. limosus* detection with a new real-time PCR assay with a Taqman probe (Applied Biosystems, Foster City, CA, USA), that targets the 16S rRNA gene, has been developed and compared with the culture isolation. Primers *ill*d (5'-TAATACGAAGGGGGCAAGCGT-3') and *ill*r (5'-CACCTCTCTTGGATT CAAGC-3') and probe *il*Probe (6FAM-GGTTCGTTGCGTCAGATGTGAAAG-TAMRA), which were used in this study, were designed on the basis of multisequence alignment of all *I. limosus* 16S rDNA sequences available in the GenBank database.

To confirm specificity, the primers and probe were checked by using the BLAST program (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and also by using suspension of several bacteria recovered habitually in patients with CF. For sensitivity of the Taqman PCR assay (Applied Biosystems), the minimal CFU detectable was 2 CFU/PCR. From January 2006 through June 2007, 365 sputum samples recovered from 84 children and 61 adults with CF and 71 sputum samples recovered from 54 patients without CF were screened blindly for *I. limosus*. By using our real-time PCR, we detected 9 *I. limosus*-positive samples from 4 patients with CF (Table); 8 of these samples were also culture positive. However, all sputum samples from patients without CF were negative. In 1 patient (Table, case 17), *I. limosus* was detected by using real-time PCR 3 months before the culture was positive. Retrospectively, the patient's medical file was rechecked and his clinical respiratory condition worsened briefly at that

stage, which indicates an infection by this bacterium. Thus, in our study, the incidence of *I. limosus* was 2.8% (4.9% for adults with CF and 1.2% for children with CF). The incidence of *Burkholderia cepacia* complex during the same period and in the same patients was 2.1% (3 adults with CF were positive, data not shown).

The genus *Inquilinus* belongs to the α -*Proteobacteria*; the genus *Azospirillum* is the most closely related bacteria (2). This cluster of bacteria contains several strains that are able to grow under saline conditions and in biofilms (8,9). The mucoid phenotype of *I. limosus* may contribute to its colonization and resistance to many antimicrobial drugs. Recently, the exopolysaccharides (EPS) produced by *I. limosus* were studied. The authors indicated that *I. limosus* produces mainly 2 EPSs that exhibit the same charge per sugar residue present in alginate, the EPS produced by *Pseudomonas aeruginosa* in patients with CF. This similarity may be related to common features of the EPS produced by these 2 opportunistic pathogens that are related to lung infections (10). Transmission of *I. limosus* between patients with CF is not known, but in the report from Chiron et al., 1 of the 5 patients with *I. limosus* had a brother who had never been colonized with this bacterium despite living in the same home (5). Schmoltdt et al. reported that 3 patients were treated in the same outpatient CF clinic during overlapping time periods and each patient was infected/colonized by an individual *I. limosus* clone, which suggests that there was no transmission among these patients (4). This bacterium has been recovered mainly from sputum of adolescents (mean age 17 ± 6.47 years, range 8–35), except in our study with a 2-year-old boy, which suggests that this emerging bacterium may be hospital acquired, as recently suggested (7). Because this bacterium is multiresistant to several antimicrobial drugs, particularly colistin, which is widely

Table. Clinical and epidemiologic features of cystic fibrosis (CF) patients with *Inquilinus limosus**

Case no.	Age, y/sex	Lung transplant	Positive samples	Clinical manifestation, first isolation	Growth on MacConkey agar	Growth on selective agar (d)	Phenotypic identification†	Other associated pathogens	Reference
1	22/F	Yes	Lung explant, BAL, sputum	Pneumonia	Poor	ND	AR	PA, PM	(1)
2	17/M	No	Sputum	Stable	No	Yes (6 d)	SP	SA, PA, CA	(3)
3	14/F	No	Sputum	Stable	No	Yes (5 d)	SP	PA, AF, CA	(3)
4	12/M	No	Sputum	Stable	ND	Yes (ND)	SP	PA, SM, SA, AX	(5)
5	13/F	No	Sputum	Exacerbation	ND	Yes (ND)	SP	PA, SA, AF	(5)
6	8/M	No	Sputum	Stable	ND	Yes (ND)	SP	PA	(5)
7	10/M	No	Sputum	Stable	ND	Yes (ND)	SP	None	(5)
8	18/M	No	Sputum	Exacerbation	ND	Yes (ND)	AR	PA, SA, AF	(5)
9	16/F	No	Sputum	Severe exacerbation	ND	ND	PA	PA	(4)
10	19/M	No	Sputum	Stable	ND	ND	ND	PA	(4)
11	17/F	No	Sputum	Exacerbation	ND	ND	ND	PA, CA, AF	(4)
12	20/F	No	Sputum	Exacerbation	ND	ND	ND	PA, SA, CA, AF	(4)
13	17/F	No	Sputum	Stable	ND	ND	ND	PA, SA, SM, CA, AF	(4)
14	35/M	No	Sputum	Respiratory decline	ND	ND	PA	PA, SM, SMA	(4)
15	17/F	No	Sputum	Stable	No	Yes (4 d)	SP	CA	This study
16	2/M	No	Sputum	Productive cough	No	Yes (3 d)	SP	SA, HI	This study
17	21/M	No	Sputum	Exacerbation	No	Yes (3 d)	AR	PA, AF	This study
18	15/M	No	Sputum	Fever and thoracic pain	No	Yes (3 d)	AR	SA	This study

*BAL, bronchoalveolar lavage; ND, not determined; AR, *Agrobacterium radiobacter*; PA, *Pseudomonas aeruginosa*; PM, *Proteus mirabilis*; SP, *Sphingomonas paucimobilis*; SA, *Staphylococcus aureus*; CA, *Candida albicans*; AF, *Aspergillus fumigatus*; SM, *Stenotrophomonas maltophilia*; AX, *Achromobacter xylosoxidans*; SMA, *Serratia marcescens*; HI, *Haemophilus influenzae*.

†Phenotypic identification was obtained by using the BIOLOG GN MicroPlate assay (BIOLOG Inc., Hayward, CA, USA) for case 1 and the API 20NE kit system (bioMérieux, Marcy l'Etoile, France) for cases 2–8 and 15–18.

used for treatment for *P. aeruginosa* colonization (as was the case for our 4 patients), we hypothesize that this bacterium is selected during the evolution of the disease.

We have developed a real-time PCR molecular method that is faster and easier than amplification-sequencing for prompt detection and accurate identification of *I. limosus* with good specificity and sensitivity. By using this screening assay, we identified 4 additional cases of patients with CF who were also infected with this bacterium, including a 2-year-old child. In addition, by using this technique, we were able to detect *I. limosus* in a patient with deteriorated respiratory function 3 months before the culture-based isolation, indicating that a low bacterial load, insufficient for being isolated in culture, can be detected by PCR in the lower respiratory tract of patients with CF.

This work was partly funded by the French Association Vaincre La Mucoviscidose.

Mr Bittar is a PhD student at URMITE UMR, Faculty of Medicine, Marseille. His research interest is detection and description of new or emerging pathogens in cystic fibrosis patients.

**Fadi Bittar,* Anne Leydier,†
Emmanuelle Bosdure,†
Alexandre Toro,*
Martine Reynaud-Gaubert,‡
Stéphanie Boniface,‡
Nathalie Stremler,†
Jean-Christophe Dubus,†
Jacques Sarles,† Didier Raoult,*
and Jean-Marc Rolain***

*Université de la Méditerranée, Marseille, France; †Hôpital Timone, Marseille, France; and ‡Hôpital Sainte Marguerite, Marseille, France

References

1. Pitulle C, Citron DM, Bochner B, Barbers R, Appleman MD. Novel bacterium isolated from a lung transplant patient with cystic fibrosis. *J Clin Microbiol.* 1999;37:3851–5.
2. Coenye T, Goris J, Spilker T, Vandamme P, Lipuma JJ. Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. *J Clin Microbiol.* 2002;40:2062–9.
3. Wellinghausen N, Essig A, Sommerburg O. *Inquilinus limosus* in patients with cystic fibrosis, Germany. *Emerg Infect Dis.* 2005;11:457–9.
4. Schmoltdt S, Latzin P, Heeseemann J, Griese M, Imhof A, Hogardt M. Clonal analysis of *Inquilinus limosus* isolates from six cystic fibrosis patients and specific serum antibody response. *J Med Microbiol.* 2006;55:1425–33.
5. Chiron R, Marchandin H, Counil F, Jumas-Bilak E, Freydiere AM, Bellon G, et al. Clinical and microbiological features of *Inquilinus* sp. isolates from five patients with cystic fibrosis. *J Clin Microbiol.* 2005;43:3938–43.

6. Cooke RP, O'Neill WA, Xu J, Moore JE, Elborn JS. *Inquilinus limosus* isolated from a cystic fibrosis patient: first UK report. *Br J Biomed Sci.* 2007;64:127–9.
7. Kiratisin P, Koomanachai P, Kowwigkai P, Pattanachaiwit S, Aswapokee N, Leelaporn A. Early-onset prosthetic valve endocarditis caused by *Inquilinus* sp. *Diagn Microbiol Infect Dis.* 2006;56:317–20. Epub 2006 Jul 18.
8. Rivarola V, Castro S, Mori G, Jofre E, Fabra A, Garnica R, et al. Response of *Azospirillum brasilense* Cd to sodium chloride stress. *Antonie Van Leeuwenhoek.* 1998;73:255–61.
9. Suzuki T, Muroga Y, Takahama M, Nishimura Y. *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. *Int J Syst Bacteriol.* 1999;49:629–34.
10. Herasimenka Y, Cescutti P, Impallomeni G, Rizzo R. Exopolysaccharides produced by *Inquilinus limosus*, a new pathogen of cystic fibrosis patients: novel structures with usual components. *Carbohydr Res.* 2007;342:2404–15.

Address for correspondence: Jean-Marc Rolain, URMITE CNRS-IRD, UMR6236, Faculté de Médecine et de Pharmacie, 27 blvd Jean Moulin, 13385 Marseille CEDEX 5, France; email: jm.rolain@medecine.univ-mrs.fr

Splenic Rupture and Malignant Mediterranean Spotted Fever

To the Editor: Mediterranean spotted fever (MSF) is a *Rickettsia conorii* infection endemic to the Mediterranean. In this case, a 55-year-old man was referred to the Necker-Enfants Malades Hospital, Paris, France, for fever, myalgia, and hypotensive shock. The patient had been in Southern France (Montpellier) 6 days before symptom onset and had been bitten by a tick on the left hand. Four days later, he reported fatigue, fever (39°C), and myalgia. His medical history showed

polycystic kidney disease, which had necessitated hemodialysis and a kidney transplant. He was receiving ongoing treatment with an immunosuppressive regimen of cyclosporine, prednisolone, and tacrolimus; his baseline hemoglobin level was 15 g/dL, and creatinine level was 230 μmol/L.

At admission, the patient's temperature was 39.5°C, blood pressure 55/40 mm Hg, and heart rate 104 beats/min. Physical examination showed a diffusely tender abdomen with guarding, no hepatosplenomegaly, a nontender renal transplant, and no lymphadenopathy. Results of cardiovascular, respiratory, and neurologic examinations were unremarkable. A diffuse maculopapular cutaneous eruption was noted on the lower limbs; no eschar was detected.

Laboratory analyses showed the following values: hemoglobin 7.9 g/dL, platelet count $115 \times 10^9/L$, leukocyte count $6.7 \times 10^9/L$ (neutrophils $5.2 \times 10^9/L$, lymphocytes $1.4 \times 10^9/L$); serum creatinine 466 μmol/L, and C-reactive protein 156 mg/L. Blood cultures were negative. Serologic study results were negative for HIV, hepatitis viruses, Epstein-Barr virus, cytomegalovirus, *Legionella*, *Mycoplasma*, *Coxiella*, *Bartonella*, *Leishmania*, and *Toxoplasma* spp. Serologic testing obtained at day 1 was negative for spotted fever group (SFG) rickettsiosis.

A computed tomographic scan showed hemoperitoneum secondary to a ruptured subcapsular splenic hematoma (online Appendix Figure, available from www.cdc.gov/EID/content/14/6/995-appG.htm), and an emergency splenectomy was performed. Histopathologic evaluation of the spleen showed white pulp atrophy; the red pulp indicated congestion and ill-defined nodules, varying in size and comprising macrophages, polymorphonuclear neutrophils, and necrotic cells (Figure, panels A, B). Skin biopsy of the macular eruption on day 2 demonstrated a leukocytoclastic vasculitis with nonocclusive luminal

thrombi in the dermal capillaries (Figure, panel C).

Universal 16S rRNA gene PCR amplification on spleen and skin tissue samples and direct sequencing identified an *R. conorii*-specific 16S rRNA sequence match. We confirmed this by using primers for *gltA* and *ompA* specific for *R. conorii*. Immunohistochemical staining demonstrated *Rickettsia* in endothelial cells and macrophages in the spleen and skin (Figure, panels D–F). Blood culture, skin biopsy specimens, and splenic tissue cultures were subsequently *R. conorii* positive. Doxycycline therapy (100 mg intravenously twice a day) was instituted at day 2 because rickettsiosis was suspected. The patient dramatically improved within 72 hours and remained well 36 months after diagnosis.

MSF is a rickettsiosis belonging to the tick-borne SFG caused by *R. conorii*, an obligate intracellular bacteria transmitted by the dog tick *Rhipicephalus sanguineus*. Endemic to Mediterranean countries, MSF generally results in a benign febrile illness accompanied by a maculopapular rash, myalgia, and local black eschar at a tick bite inoculation site. A minority of persons seeking treatment display a malignant form, which results from disseminated vasculitis associated with increased vascular permeability, thrombus-mediated vascular occlusion, and visceral perivascular lymphohistiocytic infiltrates (1). Focal thrombi have been identified in almost all organs of patients with fatal cases. Manifestations of MSF include neurologic involvement, multi-organ failure, gastric hemorrhage, and acute respiratory distress syndrome; the case-fatality rate is 1.4%–5.6%.

Splenic rupture has been reported in the course of infection with several microbial agents, including Epstein-Barr virus (2), HIV, rubella virus, *Bartonella* spp. (3), *Salmonella* spp., mycobacteria (4), and *Plasmodium* spp. (5). Splenomegaly as a result of MSF has also been documented previously (6);