JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2005, p. 3938–3943 Vol. 43, No. 8
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.8.3938–3943.2005

Clinical and Microbiological Features of *Inquilinus* sp. Isolates from Five Patients with Cystic Fibrosis

Raphaël Chiron,[1] Hélène Marchandin,[2] François Counil,[3] Estelle Jumas-Bilak,[4] Anne-Marie Freydière,[5] Gabriel Bellon,[6] Marie-Odile Husson,[7] Dominique Turck,[8] François Brémont,[9] Gérard Chabanon,[10] and Christine Segonds[10]*

Centre de Ressources et de Compétences pour la Mucoviscidose (CRCM), Service des Maladies Respiratoires,[1] Laboratoire de Bactériologie,[2] CRCM, Service de Pédiatrie, Hôpital Arnaud de Villeneuve,[3] and Laboratoire de Bactériologie-Virologie, Faculté de Pharmacie,[4] Montpellier, Laboratoire de Bactériologie[5] and CRCM, Service de Pédiatrie, Hôpital Debrousse,[6] Lyon, Laboratoire de Bactériologie-Hygiène, Hôpital Calmette,[7] and CRCM, Hôpital Jeanne de Flandre,[8] Lille, and CRCM, Service de Pneumologie-Allergologie, Hôpital des Enfants,[9] and Observatoire Burkholderia cepacia, Laboratoire de Bactériologie-Hygiène, Hôpital Rangueil, Toulouse,[10] France

Received 24 February 2005/Returned for modification 10 April 2005/Accepted 29 April 2005

Patients with cystic fibrosis (CF) may be colonized with unusual gram-negative bacilli whose identification is difficult and clinical impact unclear. We describe the clinical and microbiological features of five colonizations with organisms belonging to the recently described genus *Inquilinus* in CF patients. Isolates were identified from *Burkholderia cepacia* selective medium by means of 16S rRNA analysis. All of them were resistant to colistin, penicillins, cephalosporins, and monobactams but exhibited a remarkable susceptibility to imipenem. One of the five patients was transiently colonized with a nonmucoid isolate, whereas the four other patients were persistently colonized over the period of follow-up (8 to 21 months) with mucoid isolates. Pulsed-field gel electrophoresis of SpeI-digested genomic DNA was powerful for strain genotyping and demonstrated the clonality of *Inquilinus* sp. colonization for the two patients tested. Clinical evolution after the onset of *Inquilinus* was heterogeneous, but for at least one patient the lung function worsened and eradication of *Inquilinus* sp. was unsuccessful despite several imipenem courses. Finally, *Inquilinus* spp. may represent a new threat for CF patients due to their mucoid characteristic, their multiresistant pattern to antibiotics, and their ability to persist in the respiratory tract.

The novel genus *Inquilinus* was described in 2002 among a collection of unusual gram-negative organisms recovered from patients with cystic fibrosis (CF) by use of a polyphasic taxonomic analysis (4). Seven of the described isolates were classified in the novel species *Inquilinus limosus*, and one isolate was an unnamed species. The habitat, prevalence, pathogenic potential, interpatient transmissibility, as well as antimicrobial susceptibility of these species are unknown to date. *Inquilinus* sp. has been recovered from the sputum of five CF patients receiving care in four French CF centers located in different regions and identified by means of 16S rRNA analysis. In this report, we describe the clinical and microbiological features of these five colonizations.

CASE REPORTS

Five patients, four males and one female, receiving care in the CF Centers of Montpellier, Toulouse, Lille, and Lyon, France, acquired *Inquilinus* sp. at ages varying from 8 to 18 years. In all patients, the diagnosis of CF had been established on the basis of positive sweat tests (chloride concentration > 60 mEq/liter) and ΔF508 homozygous genotype. They had a pancreatic insufficiency but no diabetes mellitus. In all patients, exacerbations were defined according to Rabin et al. (9), and the Shwachman and Kulczycki (12) and Brasfield et al. (2) scores were used to assess clinical and radiological status, respectively. The main clinical and microbiological characteristics of each case are summarized in Table 1.

In four patients (patients 1, 2, 4, and 5), *Inquilinus* sp. was identified from at least seven sputum specimens. In patient 4, with a mild respiratory disease, *Inquilinus* sp. was not associated with any other known respiratory pathogen in the sputum, and colonization was followed by a spirometric deterioration, though without clinical signs of exacerbation; imipenem plus tobramycin and steroids slightly improved his lung function (forced vital capacity rose from 73 to 77% and forced expiratory volume in 1 second from 63 to 73%). This improvement was transient, and in the following months, the patient deteriorated despite adequate antibiotic courses against *Inquilinus* sp. according to antibiograms. The remaining three patients were colonized with several other pathogens. Patient 1 was clinically stable at the time of the first isolation of *Inquilinus* sp. and did not receive any specific antibiotic treatment; no change of the pulmonary function tests was observed during the 21 following months. Patients 2 and 5, with an advanced respiratory disease, were in an exacerbation phase at the time of the first isolation of *Inquilinus* sp. Patient 2 presented several exacerbations in the period of follow-up, one of which was

* Corresponding author. Mailing address: Observatoire Burkholderia cepacia, Laboratoire de Bactériologie-Hygiène, Hôpital Rangueil, TSA50032, 31059 Toulouse cedex 9, France. Phone: 33 5 61 32 21 55. Fax: 33 5 61 32 26 20. E-mail: segonds@cict.fr.

TABLE 1. Clinical and microbiological characteristics of five colonizations with *Inquilinus* in cystic fibrosis patients[a]

Patient characteristic	Datum for patient:				
	1 (Montpellier)	2 (Toulouse)	3 (Lille)	4 (Lyon)	5 (Lyon)
Sex	M	F	M	M	M
Age at CF diagnosis	6 yr	Birth	17 months	Birth	Birth
Age at first *P. aeruginosa* colonization (yrs)	11	12	2	5	12
Therapeutic requirements within the year preceeding the acquisition of *Inquilinus*					
No. of respiratory exacerbations	1	3	2	3	4
No. of i.v. antipseudomonal antibiotic courses	1	3	1	0	2
Other treatments	None	Antifungal agents	Azithromycin	None	Oral steroids (ABPA)
Age at first isolation of *Inquilinus* (yrs)	12	13	8	10	18
Pathogens associated with *Inquilinus* sp.	PA, SA, SM, AX	PA, SA, AF	PA	None	PA, SA, AF
Clinical status at first *Inquilinus* sp. colonization	Stable	Exacerbation	Stable	Stable but followed by spirometric deterioration	Exacerbation
No. of specimens positive with *Inquilinus* sp./total no. of specimens (duration of follow-up)	7/7 (21 mo)	7/9 (15 mo)	1/6 (15 mo)	9/10 (12 mo)	7/10 (8 mo)
Patient status before/after *Inquilinus* sp. recovery					
Shwachman clinical score	85/85	55/45	95/95	75/70	70/70
Brasfield radiological score	2/2	9/6	2/2	5/6	7/9
FVC (% pred)	99/99	85/63	116/120	82/73	91/86
FEVI (% pred)	94/93	74/51	103/120	75/63	58/43
BMI (kg/m^2)	15.8/15.9	15/15	16.1/16.3	14.1/14.2	17/16.5

[a] Abbreviations: M, male; F, female; i.v., intravenous; ABPA, allergic bronchopulmonary aspergillosis; PA, *P. aeruginosa*; SA, *Staphylococcus aureus*; SM, *Stenotrophomonas maltophilia*; AX, *Achromobacter xylosoxidans*; AF, *Aspergillus fumigatus*; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; BMI, body mass index.

treated with imipenem plus ciprofloxacin, according to the antibiograms of the *P. aeruginosa* and *Inquilinus* sp. isolates, and a transient disappearance of *Inquilinus* sp. from the sputum cultures was observed. She now has better lung function and remains stable despite moderate to severe lung disease and persistent colonization with *Inquilinus* sp. In patient 5, allergic bronchopulmonary aspergillosis was diagnosed and treated with oral steroids 5 months before *Inquilinus* sp. colonization. Steroids failed to improve his lung function, and treatment was discontinued due to side effects. *Inquilinus* sp. was demonstrated 1 month later when the patient was deteriorating. A specific treatment against *Inquilinus* sp. was considered. The patient experienced three acute exacerbations, but imipenem plus amikacin or gentamicin failed to cause significant improvement. The last exacerbation was marked by a spontaneously cured pneumothorax. Then the patient deteriorated without clinical benefit of antibiotic courses, and he remains unstable. His brother, also affected by CF, has never been colonized with *Inquilinus* sp. despite living in the same home.

In one patient with mild respiratory disease (patient 3), *Inquilinus* sp. was isolated only once, in association with *Pseudomonas aeruginosa*. His clinical status was stable. This patient did not receive any specific treatment against *Inquilinus* sp., and this organism has thus far not been recovered again, even during exacerbation periods.

MATERIALS AND METHODS

Bacterial strains. The first clinical isolate from each patient was analyzed and compared with the type strain, *Inquilinus limosus* LMG 20952^T. In addition, successive isolates recovered from patients 1 and 2 were collected in order to assess the chronicity of the lung colonization by means of genotyping.

Phenotypic tests. Colonial morphology was observed on various culture media: Mueller-Hinton, bromocresol purple lactose, blood-supplemented Columbia (BCA), and chocolate Polyvitex agars (bioMérieux, Marcy-l'Etoile, France). Growth at 30°C and 42°C was tested using BCA. Each strain was plated on selective media recommended for the analysis of the sputum of patients with cystic fibrosis, i.e., cetrimide agar for *P. aeruginosa* and oxidation-fermentation-polymyxin-bacitracin-lactose (OFBPL) (15) and *P. cepacia* medium (PC) (5), obtained from AES, Combourg, France, and *Burkholderia cepacia* selective agar (BCSA) (7), kindly provided by bioMérieux. The selective media were observed after 24, 48, and 72 h at 37°C. Biochemical characterization was performed using oxidase and ONPG *o*-nitrophenyl-β-D-galactopyranose unitary tests (Becton Dickinson, Le Pont-de-Claix, France) as well as API 20 NE and ID 32 GN strips inoculated and read according to the recommendations of the manufacturer (bioMérieux).

Antimicrobial susceptibility testing. The antibiotic susceptibility of the bacterial strains was determined using the disk diffusion test according to the recommendations of the SFM antibiogram committee (8) for gram-negative nonfermenters. The inoculum suspension was swabbed on Mueller-Hinton agar (bioMérieux). The following disks (Bio-Rad, Marnes-la-Coquette, France) were tested: ticarcillin (75 μg), ticarcillin-clavulanic acid (75/10 μg), piperacillin (75

TABLE 2. Cultural characteristics of the five *Inquilinus* isolates of this study compared to those of the type strain of *Inquilinus limosus*

Characteristic or test	Clinical isolates (n = 5)	Strain LMG 20952^T
Mucoid colonies	4/5	Yes
Growth tests		
Blood Columbia agar 30°C	5/5	Yes
Blood Columbia agar 42°C	5/5	Yes
Cetrimide agar 37°C	0/5	No
B. cepacia media 37°C		
OFBPL	5/5	Yes
PC	5/5	Yes
BCSA	0/5	No

μg), piperacillin-tazobactam (75/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), aztreonam (30 μg), imipenem (10 μg), kanamycin (30 IU), gentamicin (15 μg), tobramycin (10 μg), amikacin (30 μg), colistin (50 μg), pefloxacin (5 μg), ciprofloxacin (5 μg), doxycycline (30 IU), trimethoprim-sulfamethoxazole (1.25/23.75 μg), rifampin (30 μg), and fosfomycin (50 μg). The MIC of gentamicin was tested for gentamicin-resistant strains, in view of the results for growth on BCSA, by use of the E-test (AB Biodisk, Solna, Sweden) according to the recommendations of the manufacturer.

ARDRA. Amplified 16S rRNA restriction analysis (ARDRA) was performed as previously described (10), and the restriction profiles obtained with AluI, CfoI, DdeI, and MspI were compared with those obtained with the type strain of *Inquilinus limosus* and representative strains of other species of nonfermentative gram-negative bacilli involved in CF (10, 11).

16S rRNA sequencing. One isolated colony was suspended in 50 μl of sterile distilled water, and the DNA was rapidly extracted by a method involving boiling and freezing. 16S rRNAs were selectively amplified by PCR as previously described (3), using 27f (5′-GTGCTGCAGAGAGTTTGATCCTGGCTCAG-3′; positions 8 to 36 [*Escherichia coli* numbering]) as the forward primer and 1492r (5′-CACGGATCCTACGGGTACCTTGTTACGACTT-3′; positions 1478 to 1508 [*E. coli* numbering]) as the reverse primer. PCR products were directly sequenced on an Applied Biosystems automatic sequencer (Genome Express, Meylan, France) by using primer 63f (5′-CAGGCCTAACACATGCAAGTC-3′; positions 43 to 63 [*E. coli* numbering]). Partial 16S rRNA sequences of about 600 bp were compared with sequences deposited in the GenBank database using the BLAST (Basic Local Alignment Search Tool) program (1).

PFGE. The isolates were grown overnight on Mueller-Hinton agar, and bacteria were suspended in Tris-EDTA buffer and adjusted to an optical density of 1.3 at 650 nm. Genomic DNAs were prepared in agarose plugs as previously described (6). DNAs were digested with 40 U of SpeI (New England Biolabs) during 5 h at 37°C. Pulsed-field gel electrophoresis (PFGE) was then performed at 10°C with a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA) in a 1% agarose gel in Tris-borate-EDTA buffer (TBE 0.5×). The running parameters were as follows: voltage, 4.5 V/cm; angle, 120°; pulse ramp, 70 to 10 s for 40 h. The gels were stained with ethidium bromide and photographed under UV light. A lambda ladder (successively larger concatemer of 48.5-kb DNA fragments) was used as a molecular size marker. DNA banding patterns were compared by visual inspection and interpreted according to the criteria of Tenover et al. (13).

RESULTS

Phenotypic analysis. Growth characteristics are summarized in Table 2, and biochemical features in Tables 3 and 4. A milky mucoid aspect was observed for four of the five strains and was more pronounced on bromocresol purple lactose and Mueller-Hinton agars than on BCA or chocolate media. All strains grew at 42°C. Whereas all strains grew on OFBPL and PC *B. cepacia* selective media, none of them grew on BCSA. The API 20 NE strip classified the clinical isolates either as *Sphingomonas paucimobilis* or as *Agrobacterium radiobacter* and the type strain as *S. paucimobilis*. The ID 32 GN strip led to uninterpretable results, except for two isolates, for which *Pseudomonas fluorescens* or *Burkholderia cepacia* and *Klebsiella* species were proposed. Antimicrobial susceptibility profiles are summarized in Table 5. All isolates were fully resistant to penicillins and cephalosporins, kanamycin, tobramycin, fosfomycin, colistin, doxycycline, and co-trimoxazole (absence of zone of growth inhibition) and susceptible to imipenem, with remarkably huge zone diameters (40 to 55 mm), and ciprofloxacin. Rifampin and gentamicin exhibited a variable activity. The MICs of gentamicin for the two gentamicin-resistant strains, i.e., the type strain and one of the five clinical isolates, were 8 mg/liter.

16S rRNA analysis. All isolates exhibited the same AluI, DdeI, and MspI ARDRA profiles as the type strain of the species; three isolates exhibited the same CfoI restriction pattern as the type strain, whereas two isolates (from patient 2 [Toulouse] and patient 5 [Lyon]) exhibited a peculiar CfoI restriction pattern (Fig. 1). These profiles were unequivocally different from those obtained with other colistin-resistant organisms isolated in CF, such as *Burkholderia* spp., *Ralstonia* spp., *Pandoraea* spp., or *Ochrobactrum intermedium*. Analysis of partial 16S rRNA sequences obtained for the first strain isolated for each patient revealed that the five strains shared 99.6 to 100% sequence identity with each other and with the sequences deposited in databases for the eight representative strains of the genus *Inquilinus*, including the type strain, *I. limosus* AU0476^T (the same strain as LMG 20952^T) (GenBank

TABLE 3. Identifications obtained from the API 20 NE and 32 GN strips for the five *Inquilinus* isolates of this study and for the type strain of *Inquilinus limosus*

Test	Identification of clinical isolate from patient:					Strain LMG 20952^T
	1 (Montpellier)	2 (Toulouse)	3 (Lille)	4 (Lyon)	5 (Lyon)	
API 20 NE strip						
Numerical profile	0027744	0467544	0427544	0427544	1467744	0425544
Taxon (% identification)	*S. paucimobilis* (54.7) *A. radiobacter* (36.7)	*S. paucimobilis* (53.2) *A. radiobacter* (46.7)	*S. paucimobilis* (69.4) *A. radiobacter* (30.5)	*S. paucimobilis* (69.4) *A. radiobacter* (30.5)	*A. radiobacter* (99.9) *S. paucimobilis* (0.1)	*S. paucimobilis* (98.4)
API 32 GN strip						
Taxon (% identification)	*P. fluorescens* (58.3) *B. cepacia* (41.5)	Undetermined	Undetermined	Undetermined	*Klebsiella* spp. (85.5) *K. pneumoniae* (13.5)	Undetermined

TABLE 4. Biochemical characteristics of the five *Inquilinus* isolates of this study compared to those of the type strain of *Inquilinus limosus*[a]

Characteristic	No. of positive tests/no. of interpretable tests for clinical isolates (n = 5)	Strain LMG 20952^T
Oxidase	5/5	+
β-Galactosidase	5/5	+
Nitrate reduction	1/5	−
Indole production	0/5	−
Arginine dihydrolase	0/5	−
Urease	0/5	−
Esculin	4/5	−
Gelatin	0/5	−
Assimilation tests [substrate (technique)]		
Sodium acetate (ID 32 GN)	2/5	−
Adipic acid (API 20 NE)	0/5	−
L-Alanine (ID 32 GN)	5/5	+
L-Arabinose (API 20 NE)	5/5	+
L-Arabinose (ID 32 GN)	4/5	+
Capric acid (API 20 NE)	0/5	−
Capric acid (ID 32 GN)	2/5	−
Trisodium citrate (API 20 NE)	0/5	−
Trisodium citrate (ID 32 GN)	2/5	−
L-Fucose (ID 32 GN)	3/4	+
Potassium gluconate (API 20 NE)	5/5	+
D-Glucose (API 20 NE)	2/5	−
D-Glucose (ID 32 GN)	5/5	+
Glycogen (ID 32 GN)	2/4	+
3-Hydroxybenzoïc acid (ID 32 GN)	0/5	−
4-Hydroxybenzoïc acid (ID 32 GN)	2/5	−
3-Hydroxybutyric acid (ID 32 GN)	5/5	+
L-histidine (ID 32 GN)	3/4	−
Inositol (ID 32 GN)	5/5	+
Itaconic acid (ID 32 GN)	0/5	−
Potassium 2-ketogluconate (ID 32 GN)	5/5	+
Potassium 5-ketogluconate (ID 32 GN)	4/5	+
Lactic acid (ID 32 GN)	3/4	+
Malic acid (API 20 NE)	5/5	+
D-Maltose (API 20 NE)	2/5[a]	−
D-Maltose (ID 32 GN)	2/4[a]	+
Sodium malonate (ID 32 GN)	2/5	+
D-Mannitol (API 20 NE and ID 32 GN)	5/5	+
D-Mannose (API 20 NE)	5/5	−
D-Melibiose (ID 32 GN)	3/5	+
N-acetyl-glucosamine (API 20 NE and ID 32 GN)	5/5	+
Phenylacetic acid (API 20 NE)	0/5	−
L-Proline (ID 32 GN)	4/4	+
Propionic acid (ID 32 GN)	2/5	−
L-Rhamnose (ID 32 GN)	2/5	+
D-Ribose (ID 32 GN)	5/5	+
Salicine (ID 32 GN)	3/5	+
L-Serine (ID 32 GN)	3/5	−
D-Sorbitol (ID 32 GN)	5/5	+
Suberic acid (ID 32 GN)	1/5	nd[b]
D-Sucrose (ID 32 GN)	2/4	+
Valeric acid (ID 32 GN)	1/5	−

[a] Discordant results between API 20 NE and ID 32 GN were observed for two isolates.
[b] nd, not determined.

TABLE 5. Antimicrobial susceptibility profiles of the five *Inquilinus* isolates of this study compared to those of the type strain of *Inquilinus limosus*

Antimicrobial agent	No. of clinical isolates (out of five) that are[a]:			Strain LMG 20952^T profile
	S	I	R	
Ticarcillin	0	0	5	R
Ticarcillin-clavulanic acid	0	0	5	R
Piperacillin	0	0	5	R
Piperacillin-tazobactam	0	0	5	R
Cefotaxime	0	0	5	R
Ceftazidime	0	0	5	R
Cefepime	0	0	5	R
Aztreonam	0	0	5	R
Imipenem	5	0	0	S
Pefloxacin	2	3	0	I
Ciprofloxacin	5	0	0	S
Kanamycin	0	0	5	R
Gentamicin	4	0	1	R
Tobramycin	0	0	5	R
Amikacin	0	1	4	R
Fosfomycin	0	0	5	R
Colistin	0	0	5	R
Doxycycline	0	0	5	R
Trimethoprim-sulfamethoxazole	0	0	5	R
Rifampin	4	0	1	S

[a] S, susceptible; I, intermediate; R, resistant.

accession number AY043374), and *Inquilinus* sp. strain AU1979 (GenBank accession number AY043375).

PFGE restriction patterns. PFGE of SpeI-restricted DNA was powerful for *Inquilinus* isolates' comparison, since digestion led to profiles displaying from about 10 to 20 DNA fragments (Fig. 2). The five patients harbored genetically unrelated isolates, including the two patients receiving care in the same CF center. Surprisingly, the PFGE pattern of the isolate recovered from patient 1 was closely related to that of strain LMG 20952^T (two DNA fragment differences). The pulsotypes obtained for successive isolates from patients 1 and 2 were indistinguishable, demonstrating that both patients were each persistently colonized with the same clone.

DISCUSSION

The initial description of the novel genus *Inquilinus* in 2002 was based on eight isolates recovered from the respiratory secretions of patients with CF; seven isolates were classified in the species *I. limosus*, whereas one isolate (strain AU1979) remained unnamed to the species level (4). The five isolates described here were also recovered from CF patients and were identified by use of ARDRA and 16S rRNA sequencing. Most phenotypic characteristics were in agreement with the initial description of the genus, with the exception of nitrate reduction, which was positive in 1/5 strains, and of the following assimilation tests, previously reported as negative: inositol, mannitol, and sorbitol. The results of some assimilation tests were strain dependent (sodium acetate, fucose, glycogen, 4-hydroxybenzoic acid, histidine, potassium 5-ketogluconate, lactic acid, sodium malonate, melibiose, propionic acid, rhamnose, salicine, serine, suberic acid, sucrose, and valeric acid) and/or technique dependent (arabinose, capric acid, citrate, and glucose). *Inquilinus* spp. are not recorded in commercial identification systems' databases, due to the recent description of the genus and the low number of characterized isolates to date; a widely used test, such as the API 20 NE strip (bioMerieux),

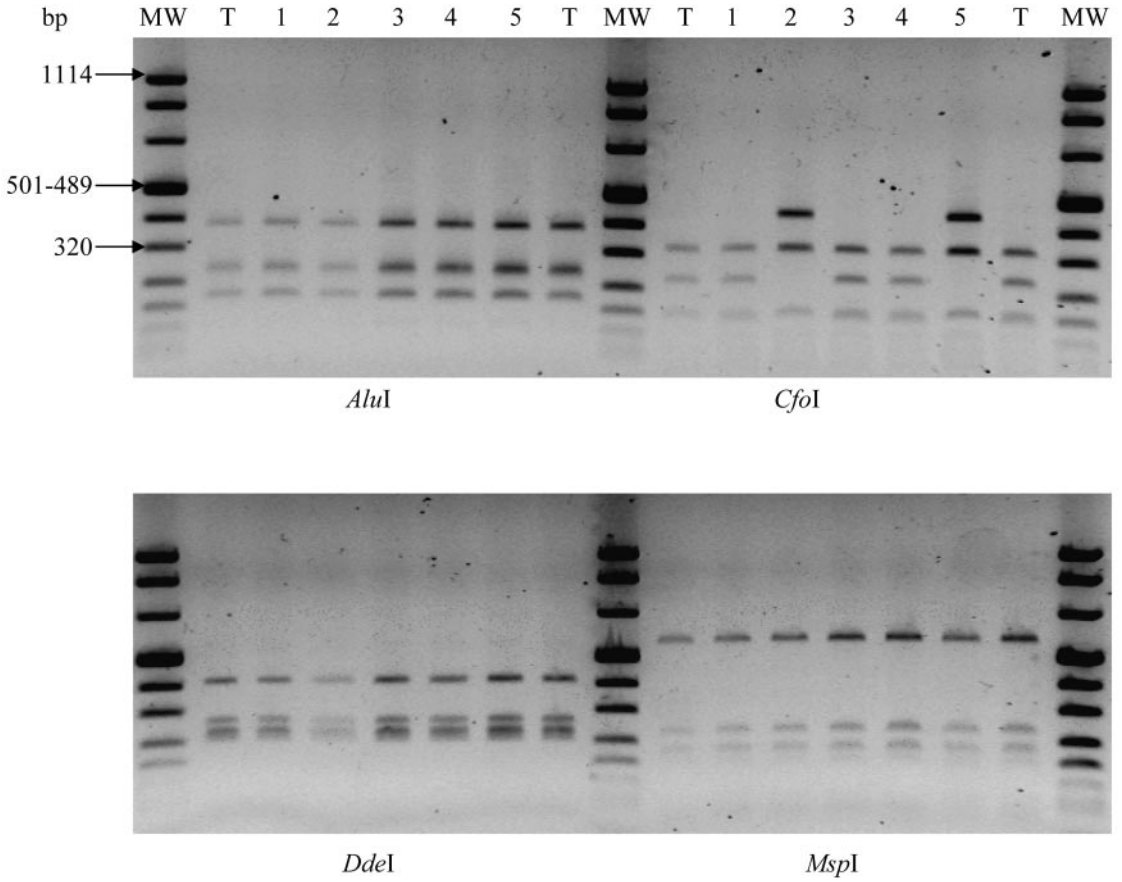

FIG. 1. ARDRA profiles of the five clinical isolates of this study compared to those of the type strain of *Inquilinus limosus*. Lanes 1 to 5, clinical isolates; lanes T, LMG 20952^T; lanes MW, molecular weight marker.

classified our isolates either as *S. paucimobilis* or as *A. radiobacter*, which was unsatisfactory given the high level of resistance to β-lactams and the morphology of colonies, since *S. paucimobilis* produces a yellow pigment. Thus, a correct identification is difficult for laboratories in which molecular methods are not available. The mucoid aspect, although it was not observed in one of the five strains, and the resistance to colistin and to all β-lactams but imipenem are useful orientation features for the clinical microbiologist. Nevertheless, these features are also consistent with *Ochrobactrum intermedium* (14).

Isolates can be recovered on colistin containing *B. cepacia* selective media except on BCSA, which also contains gentamicin (7). In the present study, one of the clinical strains and the type strain were shown to be resistant to gentamicin but nonetheless did not grow on BCSA. This is in contrast to the previously reported growth of LMG 20952^T on BCSA (4). This discrepancy may be explained by the concentration of gentamicin in BCSA (10 mg/liter), since the MIC of gentamicin for both strains was 8 mg/liter, which is consistent with at least a partial inhibition.

The 16S rRNA sequences of the five clinical isolates displayed more than 99.6% similarity to the sequences previously deposited for several strains of the genus *Inquilinus* and only 92% to the second closest genus, *Azospirillum*. Besides, they shared at least 99.6% similarity to each other, which is consistent with the intraspecies variability previously reported for

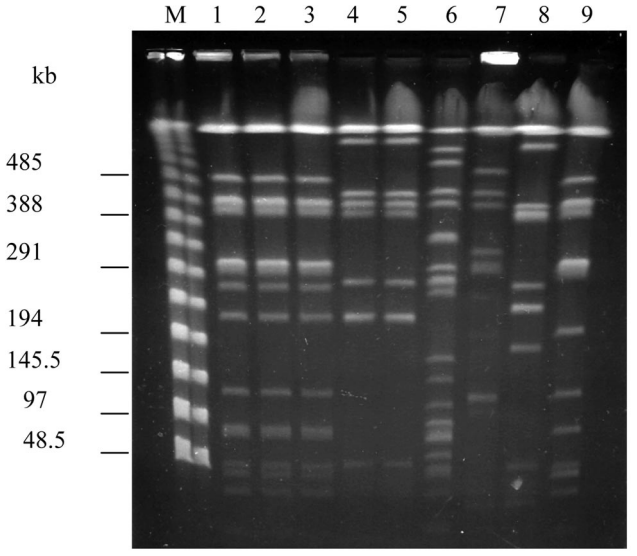

FIG. 2. PFGE of SpeI-restricted DNA for eight clinical strains of *Inquilinus* and the *I. limosus* type strain. Lane M, concatemer of phage lambda DNA (48.5 kb) as molecular weight marker; lanes 1 to 3, strains successively isolated from patient 1; lanes 4 and 5, two strains from patient 2; lanes 6, 7, and 8, strains from patients 3, 4, and 5, respectively; lane 9, *I. limosus* strain LMG 20952^T. The size of the ladder is indicated in kilobases.

seven isolates of *I. limosus*, which displayed >99.5% similarity (4). In the same study, Coenye et al. demonstrated that 16S rRNA sequencing was unable to differentiate *I. limosus* from the potential novel *Inquilinus* species represented by strain AU1979, which was individualized on the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis whole-cell protein profiles and hybridization experiments. However, the 16S rRNA sequence of strain AU1979 has been deposited as "*Inquilinus limosus* strain AU1979 16S rRNA gene, partial sequence" (GenBank accession number AY043375), which might be confusing when using genetic databases for identification purposes. Thus, further studies are needed to assess the diversity of species within the genus *Inquilinus*, and if our clinical isolates could be unequivocally identified by means of 16S rRNA sequencing to the genus level, they could not be affiliated to a definite species.

In the present study, ARDRA was also demonstrated to be a reliable alternative to sequencing for differentiation of the genus *Inquilinus* from other genera of nonfermentative gram-negative bacilli recovered from CF patients.

Four of the five patients included in this study harbored *Inquilinus* sp. over the period of follow-up (8 to 21 months). Three of them received imipenem, which failed to eradicate the organism despite in vitro susceptibility. PFGE analysis of the sequential isolates recovered from two patients over periods of 6 and 21 months confirmed that they were persistently colonized with the same strain. Interestingly, the fifth patient, from whom *Inquilinus* sp. was isolated once, harbored a nonmucoid strain, whereas the chronically colonized patients harbored mucoid strains. These observations suggest that, as previously described for *P. aeruginosa*, *Inquilinus* might be able to undergo a switch to a mucoid phenotype, leading to biofilm formation and chronic ineradicable colonization. Thus, an extensive study of the mucoid exopolysaccharide of this new organism would provide interesting insights into the colonization mechanisms.

The natural habitat of *Inquilinus* is unknown to date. All but two patients were treated in different and distant CF centers; no cross-transmission was demonstrated between the two patients monitored in the same CF center nor between one of the patients and his sibling with CF, whose sputa are *Inquilinus* negative to date. All patients were ΔF508 homozygous and presented a typical CF phenotype with pancreatic insufficiency and bronchiectasis; all but one were previously colonized by *P. aeruginosa*. The severity of the lung disease was heterogeneous. One patient was under steroid treatment, which might be considered as a risk for new pathogen acquisition. Thus, no obvious risk factor for *Inquilinus* acquisition could be identified.

The clinical impact of chronic colonization with *Inquilinus* sp. remains unclear. Nevertheless, in one patient, *Inquilinus* sp. was the only potential pathogen recovered from the sputum, and *Inquilinus* acquisition was followed by a worsening of his lung function; despite an adequate treatment with imipenem, *Inquilinus* was not eradicated and no clinical improvement was observed.

After submission of this paper, Wellinghausen et al. reported two cases of colonization with *I. limosus* in German CF patients (16). Phenotypic characteristics and antimicrobial susceptibility profiles were similar to those described in the present paper, despite a higher susceptibility to ceftazidime. The clinical impact of *I. limosus* was considered unclear, but persistent colonization was observed in one of the patients. It is noteworthy that the clinical isolates were affiliated to the species *I. limosus* without discussion of whether they could belong to the potential novel species proposed by Coenye et al.

Thus, *Inquilinus* species appear to be emerging organisms in CF patients but might be misidentified due to insufficiency of conventional biochemical testing. Their pathogenic potential, as well as their source and epidemiology, still remains unknown. However, they may represent a new threat for cystic fibrosis patients due to (i) the mucoid characteristic of the colonies, (ii) the multiresistant pattern to antimicrobial agents, and (iii) the ability to persist in the respiratory tract. Additional studies including more isolates will be needed to increase our knowledge of *Inquilinus* spp.

ACKNOWLEDGMENTS

We are very grateful to Josiane Campos for PFGE analysis, Corinne Frechou for phenotypic and ARDRA analysis, and Corinne Teyssier for 16S rRNA sequencing.

REFERENCES

1. **Altschul, S., T. Madden, A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Brasfield, D., G. Hicks, S. Soong, and R. Tiller.** 1979. The chest roentgenogram in CF: a new scoring system. Pediatrics **63:**24–29.
3. **Carlier, J., H. Marchandin, E. Jumas-Bilak, V. Lorin, C. Henry, C. Carriere, and H. Jean-Pierre.** 2002. *Anaeroglobus geminatus* gen. nov., sp. nov., a novel member of the family *Veillonellaceae*. Int. J. Syst. Evol. Microbiol. **52:**983–986.
4. **Coenye, T., J. Goris, T. Spilker, P. Vandamme, and J. LiPuma.** 2002. Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. J. Clin. Microbiol. **40:**2062–2069.
5. **Gilligan, P., P. Gage, L. Bradshaw, D. Schidlow, and B. D. Cicco.** 1985. Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol. **22:**5–8.
6. **Gouby, A., C. Neuwirth, G. Bourg, N. Bouziges, M. Carles-Nurit, E. Despaux, and M. Ramuz.** 1994. Epidemiological study by pulsed-field gel electrophoresis of an outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a geriatric hospital. J. Clin. Microbiol. **32:**301–305.
7. **Henry, D. A., M. E. Campbell, J. J. LiPuma, and D. P. Speert.** 1997. Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. J. Clin. Microbiol. **35:**614–619.
8. **Members of the SFM Antibiogram Committee.** 2003. Members of the SFM Antibiogram Committee report 2003. Int. J. Antimicrob. Agents **21:**364–391.
9. **Rabin, H., S. Butler, M. Wohl, D. Geller, A. Colin, D. Schidlow, C. Johnson, M. Konstan, and W. Regelmann.** 2004. Pulmonary exacerbations in cystic fibrosis. Pediatr. Pulmonol. **37:**400–406.
10. **Segonds, C., T. Heulin, N. Marty, and G. Chabanon.** 1999. Differentiation of *Burkholderia* species by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene and application to cystic fibrosis isolates. J. Clin. Microbiol. **37:**2201–2208.
11. **Segonds, C., S. Paute, and G. Chabanon.** 2003. Use of amplified ribosomal DNA restriction analysis for identification of *Ralstonia* and *Pandoraea* species: interest in determination of the respiratory bacterial flora in patients with cystic fibrosis. J. Clin. Microbiol. **41:**3415–3418.
12. **Shwachman, H., and L. Kulczycki.** 1958. Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period. Am. J. Dis. Child **96:**6–15.
13. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelson, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
14. **Teyssier, C., E. Jumas-Bilak, H. Marchandin, H. Jean-Pierre, J. Jeannot, G. Dusart, V. Foulongne, and M. Simeon de Buochberg.** 2003. Species identification and molecular epidemiology of bacteria belonging to *Ochrobactrum* genus. Pathol. Biol. **51:**5–12.
15. **Welch, D., M. Muszynski, C. Pai, M. Marcon, M. Hribar, P. Gilligan, J. Matsen, P. Ahlin, B. Hilman, and S. Chartrand.** 1987. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. J. Clin. Microbiol. **25:**1730–1734.
16. **Wellinghausen, N., A. Essig, and O. Sommerburg.** 2005. *Inquilinus limosus* in patients with cystic fibrosis. Emerg. Infect. Dis. **11:**457–459.