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Isolation and Characterization by Immunofluorescence, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, Western Blot, Restriction Fragment Length Polymorphism-PCR, 16S rRNA Gene Sequencing, and Pulsed-Field Gel Electrophoresis of *Rochalimaea quintana* from a Patient with Bacillary Angiomatosis

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Rochalimaea quintana **was isolated from the blood of a French human immunodeficiency virus-infected patient with bacillary angiomatosis. The isolate showed the typical growth characteristics of *Rochalimaea* species and was inert when typical biochemical testing was used. The purpose of the present work was to characterize and compare this new isolate with reference strains of *R. quintana*, *Rochalimaea vinsonii*, and *Rochalimaea henselae* by using immunofluorescence, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot (immunoblot), restriction fragment length polymorphism-PCR of the citrate synthase gene, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis. SDS-PAGE, Western blot, restriction fragment length polymorphism-PCR with *TaqI* enzyme, and 16S rRNA gene sequencing could differentiate the three *Rochalimaea* species and allowed characterization of the French isolate as *R. quintana*. However, identification of the *Rochalimaea* isolate to the species level was more easily obtained by immunofluorescence with specific murine antisera. Pulsed-field gel electrophoresis allowed differentiation of the French *R. quintana* isolate from *R. quintana* Fuller and may serve as an epidemiological tool.**

Rochalimaea quintana is the etiologic agent of trench fever, a louse-born disease, which was extensively described during World War I and World War II (19). *Rochalimaea vinsonii*, the vole agent, has never been isolated from humans (1). *Rochalimaea henselae* is responsible for bacillary angiomatosis and peliosis hepatitis in AIDS patients (22, 25). *R. quintana* has also been associated with bacillary angiomatosis (13, 32). *R. quintana* and *R. henselae* have been associated with other less-specific clinical syndromes such as self-limited febrile illness (23), bacteremia (15, 28), endocarditis (29), meningitis (15), and neurologic disorders (10, 21, 30), either in immunocompromised or immunocompetent patients. *Rochalimaea elizabethae* is the most recently recognized species and was isolated from the blood of a patient with endocarditis (4). The 16S rRNA of *R. henselae* has been amplified from the liver biopsy of an AIDS patient with peliosis hepatitis (16). Here we report the isolation of *R. quintana* from the blood of a human immunodeficiency virus-infected patient with bacillary angiomatosis. *Rochalimaea* agents are fastidious gram-negative bacteria. On the other hand, they are inert when identification using typical clinical biochemical testing is attempted. Identification has been performed by using restriction fragment length polymorphism (RFLP)-PCR of the citrate synthase gene or by sequencing the genes coding for 16S rRNA (16S rDNA). The purpose of the present work was to characterize and compare our isolate with *Rochalimaea* reference strains by using immunofluorescence, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot (immunoblot), RFLP-PCR of the citrate synthase gene, 16S rRNA sequencing, and pulsed-field gel electrophoresis (PFGE).

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MATERIALS AND METHODS

Type cultures. *R. quintana* Fuller (ATCC VR-358) and *R. vinsonii* Baker (ATCC VR-152) were obtained from G. A. Dasch (Naval Medical Research Institute, Bethesda, Md.). *R. henselae* Houston 1 (ATCC 49882) was obtained from the Centers for Disease Control. The *Rochalimaea* isolates were cultured on blood agar, at 37°C, in a 5% carbon dioxide atmosphere.

Isolation of the *Rochalimaea* agent. A French human immunodeficiency virus-infected patient with bacillary angiomatosis presented to the Purpan Hospital in Toulouse. A blood sample was collected in a sterile heparinized Vacutainer tube (Becton Dickinson, Meylan, France), and 0.1 ml of the blood was plated onto Columbia blood agar (Biomérieux, Marcy L'Etoile, France). The plates were incubated at 37°C in a 5% carbon dioxide incubator. The blood culture yielded a *Rochalimaea* agent now designated as the Toulouse isolate after 45 days of incubation. A skin biopsy was also performed. The biopsy tissue was crushed into brain heart infusion broth and then inoculated into Columbia blood agar. No growth was detectable within 60 days of incubation.

Microscopic analysis. Bacterial colonies in primary cultures and subcultures were harvested and dispensed in phosphate-buffered saline. Small aliquots of this material were placed onto microscope slides, heat fixed, and stained by the Gimenez technique (9) for microscopic analysis.

Biochemical analysis. The presence of catalase was determined by emulsifying a colony in hydrogen peroxide. The presence of oxidase was determined by using tetramethyl-*p*-phenylenediamine. Biochemical tests were performed by using typical clinical biochemical procedures and the API 20E and API 50CH systems (Pasteur, Paris, France). Since growth of the *Rochalimaea* agents is hemin dependent (6, 26), we also

used brain heart infusion broth (Biomerieux) supplemented with 10% hemin (Sigma, St. Quentin Fallavier, France) as the biochemical test medium (6).

Immunofluorescence assay. Slides were prepared as described above and used to perform immunofluorescence experiments with mouse anti-*R. quintana* and anti-*R. henselae* sera prepared in our laboratory and a goat anti-mouse immunoglobulin (Immunotech, Marseille, France). Antisera were prepared by intraperitoneal inoculation of BALB/c mice with whole organisms of the Fuller strain of *R. quintana* and *R. henselae* ATCC 49882. Mice received three weekly intraperitoneal inoculations of 0.2 ml each, and blood was collected by cardiocentesis 1 week after the last inoculation as described previously (27).

SDS-PAGE and Western blot. *Rochalimaea* species were suspended in distilled water, and the protein concentration was adjusted to 1.5 mg/ml by spectrophotometric reading at 280 nm (protein analysis program pack; Shimadzu Kyoto, Kyoto, Japan). The bacterial suspension was mixed (1:1) with Laemmli solubilizer (4% SDS, 0.125 M Tris hydrochloride [pH 6.8], 25% glycerol, 10% 2-mercaptoethanol, 0.5% bromophenol blue) (14) and boiled for 5 min. Twenty microliters of the suspension was dispensed in each well of a polyacrylamide gel. SDS-PAGE was performed with a 12.5% separating gel and a 4% stacking gel and run in a Mini-Protean II cell (Bio-Rad Laboratories, Richmond, Calif.) at 100 V for 2 h. Low-molecular-weight standards (Bio-Rad) were used to estimate the molecular weight of the separated proteins. The gel was stained with Coomassie blue R-250 (Bio-Rad).

For Western blot analysis, the polyacrylamide gel was prepared as described above and transferred to a nitrocellulose membrane in a transblot cell (Bio-Rad) at 50 V for 4 h in an ice bath. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris-HCl [pH 7.5], 500 mM NaCl, 0.1% merthiolate). Mouse antisera against *R. quintana* or *R. henselae*, the patient serum, and sera which did not react against *Rochalimaea* agents (negative controls) were tested. Each was diluted (1:50) in TBS containing 3% nonfat dry milk and incubated with the membrane overnight. After washes in TBS, the membrane was incubated for 1 h in peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) plus IgM (heavy plus light chains; Immunotech) for the mouse antisera or peroxidase-conjugated anti-human IgG plus IgM (heavy plus light chains; Immunotech) for human sera. Conjugated antibodies were diluted (1:200) in TBS (3% nonfat dry milk) before use. Nitrocellulose was washed again in TBS, and immunologic reaction was detected by colorimetric reaction (0.015% 4-chloro-1-naphthol–0.015% hydrogen peroxide in 16.7% methanol in TBS).

RFLP-PCR analysis. Bacteria were harvested from a blood agar plate, mixed with a 5% chelex solution (Bio-Rad) (5) in an Eppendorf tube, and boiled for 30 min. The suspension was centrifuged for 10 min at 12,000 × *g*, and the supernatant was transferred to a new Eppendorf tube which was kept at room temperature for 1 h. Ten microliters of the suspension was mixed with 53.5 μl of distilled water, 6 μl of 25 mM magnesium chloride (Promega, Madison, Wis.), 10 μl of Mg-free *Taq* buffer (Promega), and 10 μl of deoxynucleoside triphosphates (2% dATP, 2% dCTP, 2% dGTP, 2% dTTP [in distilled water]; Boehringer Mannheim Biochemicals, Indianapolis, Ind.), 5 μl of each component of the primer pair, and 0.5 μl of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.; 5,000 U/ml). The DNA sequences for the oligonucleotides primers RpCs.877p and RpCs.1258n were obtained from the *Rickettsia prowazekii* citrate synthase gene (33). We used the amplification conditions previously described by Regnery et al. (23), i.e., 35 cycles of denaturation (20 s at 95°C), annealing (30 s at 48°C), and extension (2 min at 60°C). The amplification products were visualized by electrophoresis of 10 μl of each sample in a 1% agarose gel. Enzymatic digestion was performed by incubating 23.5 μl of the amplified products with enzyme buffer, 10 U of endonuclease, and 0.5 μl of bovine serum albumin if necessary. Digestion was continued for 4 h at 50°C for *Taq*I and 37°C for *Alu*I and *Mse*I (New England Biolabs, Inc., Beverly, Mass.). The digested products were separated on 8% polyacrylamide gels (100 V for 4 h) which were stained with ethidium bromide and examined with a UV transilluminator. We used DNA molecular weight marker V (Boehringer Mannheim).

16s rRNA gene sequencing. The sample preparation technique was a one-step DNA extraction using Chelex 100 (5). Amplification of the 16S rRNA gene and purification and sequencing of the PCR product were carried out as described previously (7). Briefly, the 16S rRNA gene was amplified by the universal primers (5′ to 3′ orientation) forward 16S27f AAGAGTTTGATCCTGGCTCAG and reverse 16S1492r GGTTACCTTGTTACGACTT (*Escherichia coli* nomenclature) (7). PCR products were then purified by polyethylene glycol precipitation (7). The purified PCR products were sequenced by using the linear PCR procedure described by Embley (7). The products of the sequencing reactions were analyzed on standard wedge-shaped sequencing gels. An exposure time of 24 to 72 h (Amersham beta-max film) was used for the autoradiographs. The primary sequences for the *Rochalimaea* strains were confirmed by examination of base pairing in the predicted secondary structure. The sequences were compared with 16S rRNA genes contained in the GenBank release 73.0 data base by using the DNASIS program (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). The sequences were aligned with published rRNA genes of the three *Rochalimaea* species by using the ClustalV multiple-alignment program (11). This alignment was used in the DNADIST program of Phylip 3.4 (8) to calculate evolutionary distances (Knuc values) with the assumptions of Jukes and Cantor (12).

PFGE. Cultures of *Rochalimaea* species were harvested and suspended in TNE buffer (10 mM Tris [pH 8], 150 mM NaCl, 2 mM EDTA), and the bacterial suspension was added to 1 volume of Incert agarose (FMC Bioproducts, Rockland, Maine) at 42°C to form plugs in a mold apparatus. After solidification, the plugs were transferred to lysis buffer (TNE buffer, 1% SDS, 1 mg of proteinase K per ml, 0.25% Triton X-100) and incubated for 24 h twice at 50°C. Proteinase K was inactivated by incubation in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.6]) supplemented with 0.04 mg of phenylmethylsulfonyl fluoride per ml for 1 h at 50°C twice. The plugs were then washed in TE buffer and stored in 0.2 M EDTA at 4°C or equilibrated in TE buffer for 15 min twice. For enzymatic digestion, each plug was treated individually with 270 μl of 1× enzymatic buffer and 20 U of enzyme three times for 2 h at the appropriate temperature and then stored at 4°C in EPS buffer (0.5 M EDTA [pH 8], 1% *N*-lauroylsarcosine, 1 mg of proteinase K per ml) or equilibrated in 0.5× TBE buffer (pH 8; 44.5 mM Tris-HCl, 44.5 mM boric acid, 1 mM EDTA) for 30 min twice. Electrophoresis was performed in a contour-clamped homogeneous electric field system (CHEF DR II; Bio-Rad) at 14°C in 0.5× TBE. We tested several endonucleases, namely, *Sma*I (Boehringer Mannheim) and *Eag*I, *Mlu*I, *Bss*HII, and *Sal*I (New England Biolabs). Two molecular weight markers were used, namely, the Low Range PFG Marker (0.13 to 194 kb) and the Lambda Ladder PFG Marker (48.5 to 1,018 kb; New England BioLabs). Migration conditions were 5.7 V/cm with pulse times increasing from 3 to 10 s for 24 h.

Antibiotic susceptibility testing. MICs were determined for the Toulouse isolate by using the agar dilution technique. Antibiotic powders were dissolved in the solvents supplied by the manufacturers, diluted in sterile distilled water at 10 mg/ml, and then aliquoted and frozen at −80°C until use. The antibiotic concentrations tested were 0.25 to 32 μg/ml. Mueller-Hinton agar (Biomerieux) supplemented with 5% sheep blood (Biomerieux) was used as the antibiotic assay medium. Incubation conditions of 5 days, 37°C, and 5% carbon dioxide atmosphere were found to be optimal (18).

RESULTS

Case report. A 42-year-old homosexual male, who was homeless and an intravenous drug user, presented in March 1992 at the Purpan Hospital in Toulouse with a 3-month history of cutaneous lesions. He had been HIV antibody positive since 1985. On admission, he had two superficial purple cutaneous lesions on his right arm suggesting bacillary angiomatosis and was afebrile. He had no medication. A skin biopsy was performed, and he received a 1-week course of pristinamycin (2 g daily). Histologic findings of the skin biopsy tissue confirmed bacillary angiomatosis, and electron microscopy showed the presence of small rod-shaped bacteria. Warthin Starry silver staining was not performed. Three months later, he was admitted to the hospital with multiple cutaneous lesions associated with low-grade fever, weight loss, right hemiparesis, and aphasia. Computerized tomographic examination showed multiple cerebral hypodense lesions which were a few millimeters in diameter. The presumptive diagnosis of cerebral toxoplasmosis was made, and the patient was treated with sulfadiazine and pyrimethamine with no improvement. He was then treated with high-dose cotrimoxazole for 45 days without improvement of his neurologic disorders, whereas the skin lesions resolved. He died 3 weeks later in another hospital with major neurologic disorders and without a final diagnosis. An autopsy was not performed.

Growth characteristics. Blood from the febrile patient inoculated on blood agar yielded characteristic colonies visible only after the plates had been incubated for 45 days at 37°C in a 5% CO_2 atmosphere. The CFU count in the patient blood was approximately 20 to 30 colonies per ml. Primary colonies were firmly adherent. Colony formation was visible in the first subcultures after 10 to 15 days of incubation. After multiple passages, the incubation time to colony visualization decreased to 3 to 5 days, and the colonies were larger and less adherent.

Morphologic characteristics. Organisms stained by the Gimenez technique appeared as small red bacilli, with an average size of 1 to 2 μm in length and 0.5 μm in width.

Biochemical analysis. The bacterium was nonmotile and catalase and oxidase negative. It was inert when tested by typical clinical biochemical procedures. When brain heart infusion broth supplemented with 10% hemin was used as the biochemical test medium, positive assays for all of the *Rochalimaea* isolates, including our *R. quintana* isolate, included gelatinase, hippurate, and esculin hydrolysis and oxidation of ribose. Negative results were obtained from assays for decarboxylases, urease, nitrate reductase (except for the *R. henselae* strain for which the nitrate reductase test was positive), H_2S and indole production, TDA, Voges-Proskauer, and oxidation of glucose, manitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amylose, and arabinose.

Immunofluorescence assay. Murine sera obtained after immunization contained high antibody activity and specificity for the proteins of the inoculated species. *R. quintana*-induced antibodies did not react with *R. henselae* or *R. vinsonii*, and *R. henselae*-induced antibodies did not react with *R. quintana* or *R. vinsonii*. The Toulouse isolate reacted with the serum of the patient, which had titers of 50 and 200 for IgG and 25 and 50 for IgM at hospitalization and 15 days later, respectively. It reacted weakly with the mouse antiserum to *R. quintana* and did not react with the antiserum to *R. henselae*.

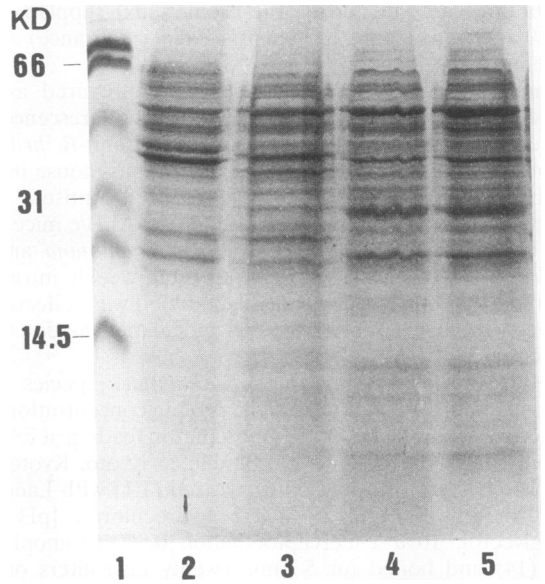

FIG. 1. Coomassie blue-stained SDS-PAGE profiles of the *Rochalimaea* strains. Lanes: 1, molecular mass standard; 2, *R. vinsonii*; 3, *R. henselae*; 4, Toulouse isolate; 5, *R. quintana*.

SDS-PAGE analysis. SDS-PAGE analysis showed distinct protein profiles for *R. henselae*, *R. quintana*, and *R. vinsonii* (Fig. 1). We found identical protein profiles with the Fuller strain of *R. quintana* and the Toulouse isolate.

Western blot. Immunoblot studies showed the development of antibodies directed at proteins of *R. quintana*, *R. henselae*, *R. vinsonii*, and the Toulouse isolate, with apparent molecular masses of 10 to 65 kDa (Fig. 2). The antibody response was stronger when using homologous mouse antisera than when using heterologous antisera. Profiles from *R. quintana* and *R. henselae* were different when either the antiserum to *R. quintana* or the antiserum to *R. henselae* was used. The Fuller strain of *R. quintana* and the Toulouse isolate gave identical profiles when antisera to either *R. quintana* or *R. henselae* or the patient serum was used. A dominant 48.5-kDa protein was present in all *Rochalimaea* species when antisera to *R. quintana* or *R. henselae* or the patient serum were used. This immunodominant 48.5-kDa protein was the only protein uniformly detected in all *Rochalimaea* species when the patient sera were tested. Control sera from five patients without *Rochalimaea* infection did not react with this 48.5-kDa protein.

RFLP-PCR analysis. We obtained positive reactions with RpCS.877p and RpCS.1258n primers for the four isolates tested, and the uncut *Rochalimaea* citrate synthase PCR products were approximately 400 bp long. We digested the four amplified DNAs with three restriction endonucleases (Fig. 3), and after migration in an 8% polyacrylamide gel, we found identical profiles for *R. quintana* Fuller and the Toulouse isolate. *Taq*I allowed identification of the three *Rochalimaea* species tested, whereas after digestion with *Mse*I, we obtained identical patterns with *R. quintana*, *R. henselae*, and the Toulouse isolate and very similar profiles were obtained after digestion with *Alu*I.

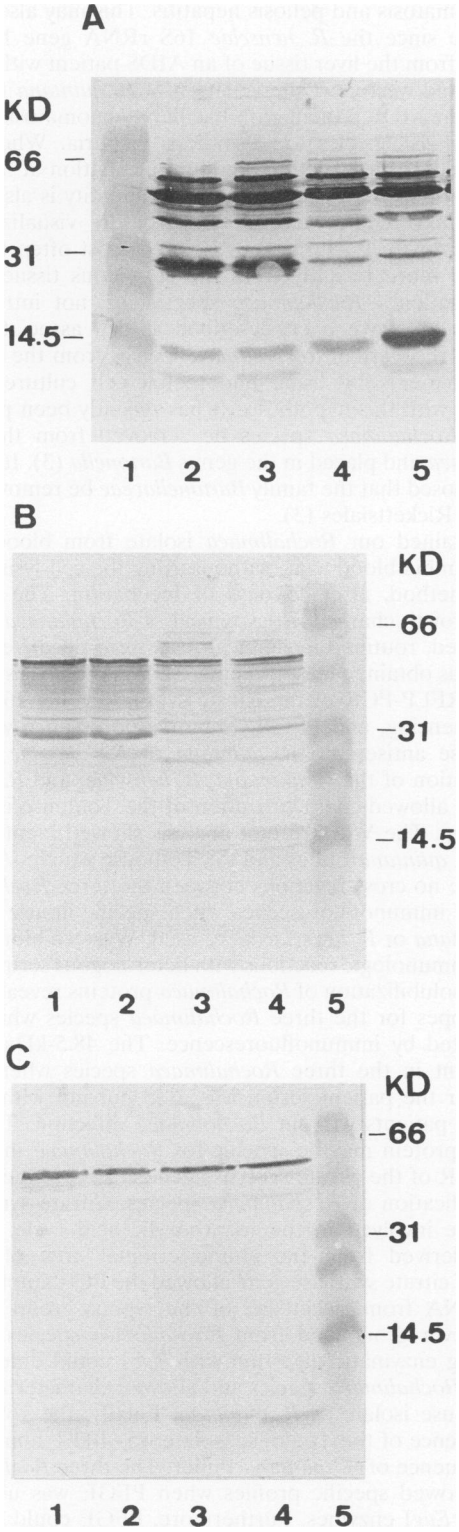

FIG. 2. Western blot of SDS-PAGE-separated proteins of *Rochalimaea* strains revealed with mouse antisera to *R. quintana* (A) and *R. henselae* (B) and with the serum of the patient (C). (A) Lanes: 1, molecular mass standard; 2, *R. quintana*; 3, Toulouse isolate; 4, *R. henselae*; *R. vinsonii*. (B and C) Lanes: 1, *R. quintana*; Toulouse isolate; 3, *R. henselae*; 4, *R. vinsonii*; 5, molecular mass standard.

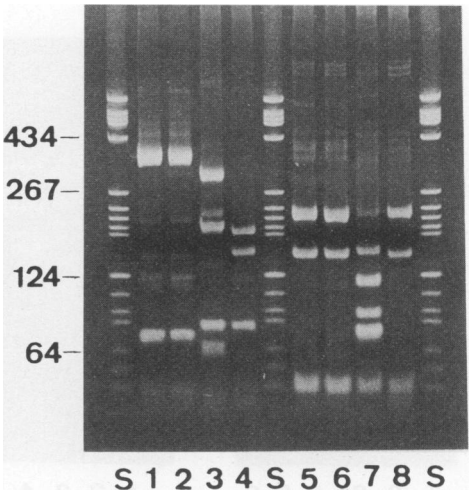

FIG. 3. Ethidium bromide-stained polyacrylamide gel of RFLP-PCR patterns of *Rochalimaea* strains. DNAs were amplified by using primers specific for the citrate synthase gene and digested by *Taq*I. (lanes: 1, *R. quintana*, 2, Toulouse isolate; 3, *R. vinsonii*; 4, *R. henselae*) and *Mse*I (lanes: 5, *R. quintana*; 6, Toulouse isolate; 7, *R. vinsonii*; 8, *R. henselae*). Lanes S indicate the DNA molecular mass marker V (in base pairs). Migration was performed in 1× TBE at 100 V for 4 h.

16S rRNA gene sequencing. By using the linear PCR, it was determined that each of the sequences comprises a continuous stretch of 1,418 bases (ranging from position 54 to position 1472 on the *E. coli* numbering system), which corresponds to approximately 95% of the total 16S rRNA primary sequence. A comparison of the sequence alignments revealed a 100% sequence similarity with the published *R. quintana* Fuller 16S rRNA sequence (23).

PFGE analysis. With knowledge of the low G+C content of the *Rochalimaea* genome (i.e., 39%) (31), we tested endonucleases with restriction sites rich in cytosine and guanine (2). Of the tested enzymes (i.e., *Eag*I, *Mlu*I, *Bss*HII, *Sma*I, and *Sal*I), two gave interesting profiles, namely, *Sma*I (CCCGGG) and *Eag*I (CGGCCG). After performing digestion with these two endonucleases, we obtained between 20 and 30 bands for each strain except for *R. vinsonii* after *Sma*I digestion. The patterns were different for *R. quintana*, *R. henselae*, and *R. vinsonii* (Fig. 4). PFGE after *Sma*I digestion showed the same profiles for *R. quintana* and the Toulouse isolate. However, after performing enzymatic digestion with *Eag*I and PFGE under the described conditions, it was possible to find small differences at 20 to 30 kb. In fact, we found three bands for each isolate, but the molecular sizes of these were 23, 23, and 28 kb for *R. quintana* and 23, 28, and 30 kb for the Toulouse isolate.

Antibiotic susceptibilities. The Toulouse *R. quintana* isolate was susceptible to most antibiotics tested (Table 1). The MICs were equivalent to those previously reported for *R. quintana* Fuller, *R. vinsonii* ATCC VR-152, and *R. henselae* ATCC 49882 (18).

DISCUSSION

We report the isolation of *R. quintana* from the blood of a French human immunodeficiency virus-infected patient with bacillary angiomatosis. To our knowledge, this is the first European isolate associated with AIDS. This patient presented with a 3-month history of skin lesions, and histologic examination of a cutaneous lesion confirmed the diagnosis of bacillary angiomatosis. An *R. quintana* isolate was grown from a blood sample.

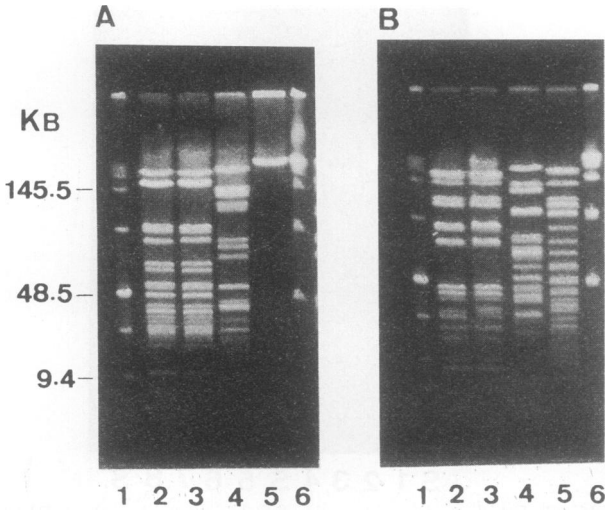

FIG. 4. PFGE of fragments generated after enzymatic digestion of *Sma*I (A) and *Eag*I (B). Lanes: 1, Low Range PFG Marker; 2, *R. quintana*; 3, Toulouse isolate; 4, *R. henselae*; 5, *R. vinsonii*; 6, Lambda Ladder PFG Marker. Migration was performed in 0.5× TBE at 14°C at 5.7 V/cm for 24 h with pulse times increasing from 3 to 10 s. Numbers to the left of panel A indicate the sizes (in kilobases) of the marker bands.

In cases of trench fever, *R. quintana* is transmitted by a louse. Its transmission in cases of bacillary angiomatosis is not known. *R. henselae* may be transmitted by cat scratches or arthropods of cats. The patient in the present study was a homosexual, intravenous-drug-using, homeless male. It is not known if he had lice at presentation. Other authors have reported the association of bacillary angiomatosis and central nervous system involvement (10, 30), with erythromycin being effective on both skin lesions and neurologic manifestations. Despite antibiotic treatment with high doses of cotrimoxazole, the patient in our study died from neurologic disorders without an etiologic diagnosis, whereas the skin lesions resolved.

In the United States, both *R. quintana* and *R. henselae* have been associated with AIDS-associated diseases such as bacillary angiomatosis and peliosis hepatitis. This may also be true in Europe since the *R. henselae* 16S rRNA gene has been amplified from the liver tissue of an AIDS patient with peliosis hepatitis and we report the isolation of *R. quintana* from the blood of an AIDS patient with bacillary angiomatosis.

TABLE 1. Antibiotic susceptibility of the Toulouse isolate

Drug	MIC (mg/liter)
Penicillin G	<0.5
Oxacillin	2.0
Amoxicillin	<0.5
Cephalothin	4.0
Cefotaxime	<0.5
Imipenem	<0.5
Erythromycin	1.0
Clarythromycin	<0.5
Azythromycin	<0.5
Clindamycin	8.0
Doxycycline	<0.5
Chloramphenicol	<0.5
Rifampin	<0.5
Trim-sulf[a]	2–10
Gentamicin	4.0
Amikacin	4.0
Ofloxacin	4.0
Ciprofloxacin	2.0
Vancomycin	8.0

[a] Trim-sulf, trimethoprim-sulfamethoxazole.

Rochalimaea species are fastidious bacteria. When blood agar is used as the isolation medium, incubation at 37°C in a 5% CO_2 atmosphere is optimal. High humidity is also useful. Isolation may require several weeks before visualization of bacterial growth. Bacteria were isolated most often from the blood and more recently from the cutaneous tissue (13) of infected patients. *Rochalimaea* species are not intracellular pathogens but have been described as cell associated (20). Enhanced recovery of *Rochalimaea* species from the blood of infected patients by using endothelial cell culture (13) is consistent with this hypothesis. It has recently been proposed that the *Rochalimaea* species be removed from the genus *Rochalimaea* and placed in the genus *Bartonella* (3). It has also been proposed that the family *Bartonellaceae* be removed from the order Rickettsiales (3).

We obtained our *Rochalimaea* isolate from blood plated onto Columbia blood agar, without using the cell-lysis centrifugation method, after 45 days of incubation. The bacteria showed growth characteristics typical of *Rochalimaea* species. As expected, routine biochemical tests were negative. Identification was obtained by serotyping, SDS-PAGE, Western blot analysis, RFLP-PCR of the citrate synthase gene, 16S rRNA gene sequencing, and PFGE. Immunofluorescence with specific mouse antisera to *R. quintana* or *R. henselae* allowed differentiation of the *R. quintana*, *R. henselae*, and *R. vinsonii* species. It allowed characterization of the Toulouse isolate as *R. quintana*. The Western blot analysis showed identical profiles for *R. quintana* Fuller and the Toulouse isolate. Although there were no cross-reactions between the three *Rochalimaea* species by immunofluorescence when specific mouse antisera to *R. quintana* or *R. henselae* were used, Western blot analysis showed immunologic reactions with heterologous serum. Thus, Laemmli solubilization of *Rochalimaea* proteins revealed common epitopes for the three *Rochalimaea* species which were not detected by immunofluorescence. The 48.5-kDa protein was present in the three *Rochalimaea* species when mouse antisera or the patient serum was used but not with control sera from patients without *Rochalimaea* infection. Thus, the 48.5-kDa protein may be specific for *Rochalimaea* infections. RFLP-PCR of the citrate synthase gene is a rapid method for the identification of *Rochalimaea* species. Citrate synthase is an enzyme involved in the tricarboxylic acid cycle. Specific primers derived from the amino-terminal area of the *R. prowazekii* citrate synthase gene allowed the PCR amplification of the DNA from rickettsiae of the typhus group and the spotted fever group and from *Rochalimaea* species. RFLP-PCR using enzymatic digestion with *Taq*I could differentiate the three *Rochalimaea* species and allowed characterization of the Toulouse isolate as *R. quintana*. Finally, the 16S rRNA gene sequence of the Toulouse isolate was 100% homologous to the sequence of *R. quintana* Fuller. The three *Rochalimaea* species showed specific profiles when PFGE was used with *Sma*I and *Eag*I enzymes. Furthermore, PFGE could differentiate the Toulouse isolate from the Fuller strain. The use of PFGE in epidemiologic investigations is becoming more and more frequent. PFGE patterns may be used to show the clonal origin of *Rochalimaea* spp. Matar et al. (17) have recently used RFLP-PCR for subtyping *R. henselae* isolates. They amplified DNA fragments which included the spacer region between the genes coding for 16S and 23S rRNA, and they performed

digestion of the amplified products with *Taq*I or *Hae*III. This method could not differentiate two *R. quintana* isolates. Since we obtained different profiles with two *R. quintana* isolates, it would also be of interest to test *R. henselae* isolates by PFGE.

We report here the isolation of *R. quintana* in a French patient with bacillary angiomatosis. An immunofluorescence assay using specific mouse antisera was the easiest method for identification of the isolate to the species level. However, D. A. Relman (24) emphasized the possibility that *R. quintana* isolates associated with bacillary angiomatosis may not be the same as those associated with trench fever. Sequencing the 16S rRNA gene may not allow such differentiation. The different profiles that we have shown by PFGE between the Toulouse isolate, which was associated with bacillary angiomatosis, and the Fuller strain of *R. quintana*, which was associated with trench fever, indicate the need for further investigation.

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