ORIGINAL ARTICLE

Bacteraemia caused by *Anaerotruncus colihominis* and emended description of the species

S K P Lau, P C Y Woo, G K S Woo, A M Y Fung, A H Y Ngan, Y Song, C Liu, P Summanen, S M Finegold, K Yuen

J Clin Pathol 2006;**59**:748–752. doi: 10.1136/jcp.2005.031773

See end of article for authors' affiliations

Correspondence to: Dr Kwok-yung Yuen, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong; hkumicro@hkucc.hku.hk

Accepted for publication 15 August 2005
Published Online First 7 February 2006

Background: *Anaerotruncus colihomonis* is a newly described bacterial genus and species isolated from the stool specimens of children. Its clinical significance, however, is unknown.
Aims: To describe a case of *A colihominis* bacteraemia identified by 16S ribosomal RNA (rRNA) gene sequencing and provide an emended description of the species.
Methods: An unidentified anaerobic bacillus (strain HKU19) that stains Gram negative was subjected to characterisation by 16S rRNA gene sequencing, G+C content determination and electron microscopy.
Results: Strain HKU19 was isolated from the blood culture of a 78-year-old woman with nosocomial bacteraemia. It was found to be an anaerobic, non-motile, pleomorphic, thin bacillus that stains Gram negative. It produces Indole and utilises glucose and mannose. Identifying the strain to the species level was not possible by conventional phenotypic tests and commercial identification systems. The G+C content of strain HKU19 was found to be 53.43 mol%. A similarity of 99.3% nucleotide identities was found between the 16S rRNA gene sequence of strain HKU19 and that of *A colihominis* WAL 14 565^T, which was isolated from a human faecal specimen. In contrast with the original description of *A colihominis*, HKU19 was found to produce occasional oval, terminal spores, although the other phenotypic characteristics matched. Spores were also occasionally observed when the two previously reported strains were re-examined.
Conclusions: Although the source of the bacteraemia in the patient cannot be determined, this report suggests that *A colihominis* is of clinical significance. Spore formation is proposed as an emended description of *A colihominis*.

The identification of anaerobic Gram-positive bacilli is often difficult in clinical microbiology laboratories. Firstly, spore formation is sometimes not obvious in bacterial isolates recovered directly from clinical specimens. Secondly, identification of bacilli by commercial kits, or analysis of cell wall fatty acids and metabolic end products by gas–liquid chromatogaphy, is difficult because of the limited database and a lack of special equipment and expertise. Thirdly, despite having Gram-positive cell walls, certain species, including many *Clostridium* species, often stain as Gram negative. As a result, the epidemiology and pathogenic potential of many members of this group of bacteria have been poorly understood. Since the recognition of the 16S ribosomal RNA (rRNA) gene as a new standard for classification and identification of bacteria,[1 2] their classification has been revised and many new species identified.[3–6]

Anaerotruncus is a newly described bacterial genus in which *A colihominis* is the only species.[7] The bacterium was first identified by phenotypic and phylogenetic studies on two isolates of an unidentified Gram-positive, anaerobic bacillus isolated from the stool specimens of two children. Owing to its loose phylogenetic association with, and phenotypic difference to, its closest relatives within the *C leptum* rRNA cluster, and, more importantly, the absence of spore formation, it was classified as a distinct genus and species. It is not known, however, whether the bacterium is only part of the human gut flora or whether it can be associated with disease. In this study, we describe the identification of an anaerobic Gram-positive bacillus associated with nosocomial bacteraemia by a combination of phenotypic and genotypic tests, including 16S rRNA gene analysis. Although the bacterium was able to produce spores, it is most likely to be a strain of *A colihominis* by phylogenetic analysis. On the basis of our findings, we propose an emended description of the species.

METHODS

Microbiological methods

Clinical data were collected prospectively. Clinical specimens were collected and handled according to standard protocols. The BACTEC 9240 blood culture system (Becton Dickinson, Maryland, USA) was used. The isolate was identified by a combination of standard conventional biochemical methods[8 9] and the Vitek (ANI; bioMerieux Vitek, North Carolina, USA), API (20A; bioMerieux Vitek, Hazelwood, Missouri, USA) and ATB Expression (rapid ID32A; bioMerieux Vitek, USA) systems. Antimicrobial susceptibility was tested by the E-test (AB Biodisk, Solna, Sweden) on Brucella blood agar plates and the results were interpreted according to the NCCLS criteria for anaerobic bacteria.[10] All tests were carried out in triplicate, with freshly prepared media on separate occasions.

Scanning electron microscopy

Scanning electron microscopy (SEM) was carried out as described in our previous publications.[11 12] Briefly, bacterial cells were settled onto a polycarbonate membrane (Nucleopore) for fixing in 2.5% glutaraldehyde. Critical dried material was coated with palladium in a BAL-TEC SCD 005 SEM coating system and examined under a Leica Cambridge

Abbreviations: rRNA, ribosomal RNA; SEM, scanning electron microscopy; Tm, melting temperature; PCR, polymerase chain reaction; rRNA, ribosomal RNA

Stereoscan 440 scanning electron microscope operating at 12 kV.

16S rRNA gene sequencing and phylogenetic characterisation

Bacterial DNA extraction, PCR amplification and DNA sequencing of the 16S rRNA gene of HKU19 were carried out with polymerase chain reaction (PCR) primers (LPW57 5′-AGTTTGATCCTGGCTCAG-3′ and LPW205 5′-CTTGTTACG ACTTCACCC-3′) and additional sequencing primers (LPW104 5′-ACTCCTACGGGAGGCAGCAGTA-3′ and LPW306 5′-TGAGATGTTGGGTTAAGT-3′; Gibco BRL, Rockville, Maryland, USA), as described previously.[13 14] The sequences of the PCR products were compared with known 16S rRNA gene sequences in the GenBank (http://www.ncbi.nlm.nih.gov) by multiple sequence alignment with the Clustal W program.[15] The phylogenetic tree was constructed using Clustal X V.1.81[16] and the neighbour-joining method with GrowTree (Genetics Computer Group, San Diego, USA).

G+C content determination

The genomic DNA G+C content was determined by thermal denaturation according to previously published protocols.[12 17] Briefly, the temperature of the genomic DNA in standard sodium citrate (0.15 M NaCl with 0.015 M sodium citrate) buffer (25 μg/ml) was increased slowly (0.5℃/min) from 25℃ and the absorbance of the solution at 260 nm was monitored continuously against a blank containing only standard sodium citrate buffer. The melting temperature (Tm) of the DNA is defined as the temperature at 50% hyperchromicity. The G+C content of the genomic DNA was calculated by the formula (G+C)% = 2.44Tm − 169.[18]

Nucleotide sequence accession number

The 16S rRNA gene sequence of HKU19 has been lodged in the GenBank sequence database under the accession number DQ002932.

RESULTS AND DISCUSSION

Patient

A 78-year-old woman was admitted to hospital because of acute exacerbation of chronic obstructive airway disease. She also had ischaemic heart disease, hypertension and gout. She was put on prolonged mechanical ventilation because of respiratory failure and secondary pneumothorax, which resolved gradually with chest drain. During the fifth week of hospitalisation, she developed fever, with no localised symptoms or signs. Her total leucocyte count was 17.18×10^9/l (neutrophils 14.11×10^9/l, lymphocytes 1.42×10^9/l, monocytes 1.11×10^9/l and basophils 0.15×10^9/l), haemoglobin level 10 g/dl and platelet count 419×10^9/l. The renal and liver function tests were within normal limits. Blood culture was performed. Empirical intravenous cefoperazone/sulbactam and metronidazole were started. On day 2 post-incubation, the anaerobic blood culture bottle turned positive with a pure culture of a pleomorphic, thin bacillus that stained Gram negative (strain HKU19). Subsequently, no bacteria were grown from repeated blood cultures. No infection was evident and the patient responded to antibiotics. Her condition was subsequently complicated by episodes of nosocomial pneumonia and gastrointestinal bleeding associated with steroid treatment over the next few weeks. The patient ultimately survived after 3 months of hospitalisation.

Phenotypic characteristics

Strain HKU19 grew on sheep blood agar as non-haemolytic, irregularly edged, grey colonies 2–3 mm in diameter after 48 h of incubation at 37℃ in an anaerobic environment. It did not grow in ambient air, ambient air supplemented with 5% CO_2 or microaerophilic conditions. Cells formed occasional oval terminal endospores, producing a drumstick-like appearance (fig 1A). The strain was non-motile. It did not produce catalase or reduce nitrate and failed to hydrolyse aesculin, gelatin or urea. It produced indole and β-gluocosidase, and used glucose and mannose but not arabinose, cellobiose, glycerol, lactose, maltose, mannitol, melezitose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose or xylose. Glucose was fermented, with the production of acid and gas. Lysine arylamidase was detected by the Vitek ANI system. The Vitek ANI system showed that it was ''unidentified'', whereas the API system (20A) (code profile 50404003) showed a match of 83.9% to *Clostridium cadaveris* and 15.5% to *C bifermentans*, and the ATB Expression system (ID32A; code profile 0010200000) showed a 75% match to *C bifermentans*, 17% to *Fusobacterium nucleatum* and 6% to *Clostridium tetani*. Analysis of metabolic end products by gas–liquid chromatogaphy from peptone–yeast–glucose broth yielded only acetic and butyric acids, but no organic acids. The isolate was sensitive to vancomycin (5 μg), but resistant to colistin (10 μg) identification discs. The minimum inhibitory concentrations of penicillin, cefotaxime, vancomycin and metronidazole were 0.094, 0.047, 0.5 and <0.016 μg/ml, respectively.

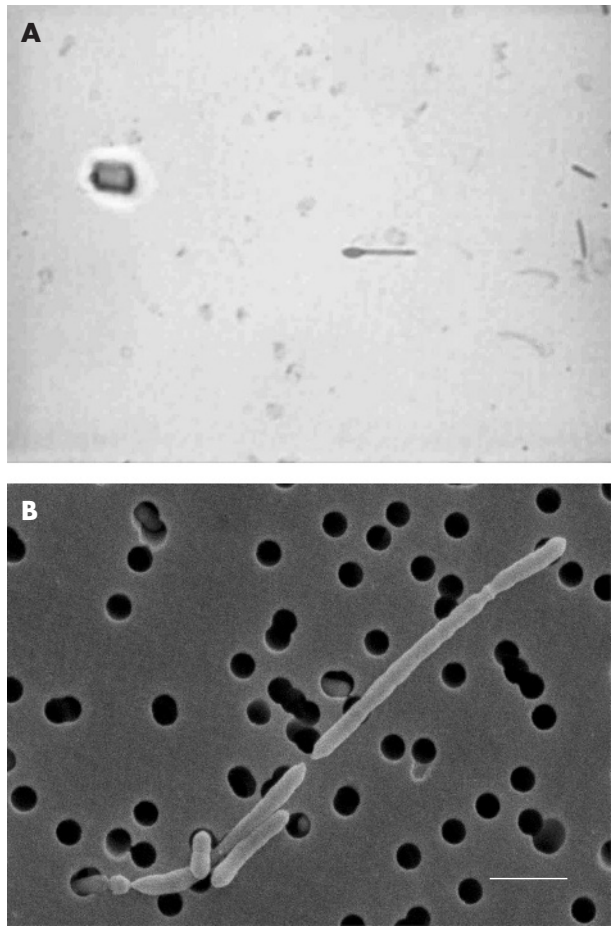

Figure 1 Spore stain of HKU19 (A) showing terminal spores (green) with a drumstick-like appearance. Scanning electron micrograph of HKU19 (B) (size bar 2 μm). Bacterial cells were bacilli, which multiplied by longitudinal division.

SEM

SEM showed that bacterial cells of HKU19 were bacilli (0.5×2−5 μm), which multiplied by longitudinal division (fig 1B).

16S ribosomal RNA gene sequencing, G+C content and phylogenetic characterisation

As conventional phenotypic tests and commercial identification kits failed to ascertain the identity of HKU19, its 16S rRNA gene was amplified and sequenced. PCR of the 16S rRNA gene showed a band at about 1400 bp. The 16S rRNA gene sequence of strain HKU19 had a 0.7% nucleotide difference from that of *A colihominis* (GenBank accession number AJ315980); a 10.2% nucleotide difference from that of *Acetanaerobacterium elongatum* (GenBank accession number AY487928); a 10.5% difference from that of *A elongatum* (GenBank accession number AY518589); a 11.5% difference from that of *C methylpentosum* (GenBank accession number Y18181); and a 12.5% difference from that of *C cellulosi* (GenBank accession number L09177; fig 2). The G+C content of strain HKU19 (mean (SD)) was 53.43 (1.09) mol%. On the basis of phylogenetic affiliation, HKU19 is a strain of *A colihominis,* which belongs to the *C leptum* suprageneric rRNA cluster. Table 1 summarises a comparison between the characteristics of HKU19 and the type strain of *A colihominis*, WAL 14 565T.

The present isolate was probably more clinically important than a contaminant because of its association with an acute febrile illness, leucocytosis, the absence of other infective foci and the patient's prompt response to antibiotics. The source of the bacteraemia was, however, unknown. The patient did not have any gastrointestinal tract disease nor had she undergone gut instrumentation. Further studies with 16S rRNA gene sequencing to characterise more clinical isolates of unidentified anaerobic Gram-positive bacilli may help better understand the epidemiology and pathogenic potential of this bacterium.

Spore formation was also evident in the two *A colihominis* strains reported previously.[7] Unlike HKU19, the originally described *A colihominis* strains were non-spore forming. After the recognition of spore formation in the strain in this study, the two recently reported strains of *A colihominis* were re-examined by both malachite green stain and phase contrast microscopy. They were also found to form occasional spores after incubation on anaerobic blood agar for 5 days. As a result, we have written an emended description of this species.

Emended description of *A colihominis*

This description is based on three strains, including the type strains WAL 14 565T and HKU19.[7] Cells are Gram-positive, thin rods of approximately 0.5×2−5 μm, that form oval, terminal spores. After 48 h of anaerobic incubation at 37℃ under an N_2/CO_2 (80:20, v/v) gas phase, colonies were 2–3 mm in diameter, grey, entire-edged, irregularly shaped, low pyramidal in profile and translucent. The pH range for growth is 5.5–11 and the growth temperature range is 36–40℃. The cells are catalase negative; nitrate is not reduced to nitrite, but indole is produced. The end products of metabolism from peptone–yeast broth are acetic and butyric acids. Aesculin, gelatin and urea are not hydrolysed. They are able to grow in peptone–yeast broth supplemented with 1% glucose, fructose, mannose or cellobiose, but they do not grow in peptone–yeast broth alone or peptone–yeast broth supplemented with arabinose, inositol, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch or xylose. With the Biolog system, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, arbutin, D-cellobiose, dextrin, D-fructose, D-galactose, D-galacturonic acid, α-D-glucose, maltose, maltotriose, D-mannose, methyl-3-D-glucose, methyl-β-D-glucose, methyl-β-D-galactoside, methyl-β-D-glucoside, palatinose, D-trehalose and turanose are used. α-Ketobutyric acid, α-ketovaleric acid, L-malic acid, pyruvic acid and pyruvic acid methyl ester are also used. Serine, L-valine, 2′-deoxyadenosine, inosine, thymidine and uridine are used for growth. With the API ZYM and Rapid ID 32A systems, acid phosphatase and indole activities are detected, but *N*-acetyl-β-glucosaminidase, alanine arylamidase, alkaline phosphatase, arginine arylamidase, arginine dehydrolase, α-arabinosidase, ester lipase C8, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, glutamyl glutamic acid arylamidase, α-mannosidase, α-fucosidase, chymotrypsin, alanine arylamidase, leucyl glycine arylamidase, phosphoamidase, trypsin, cystine arylamidase, esterase

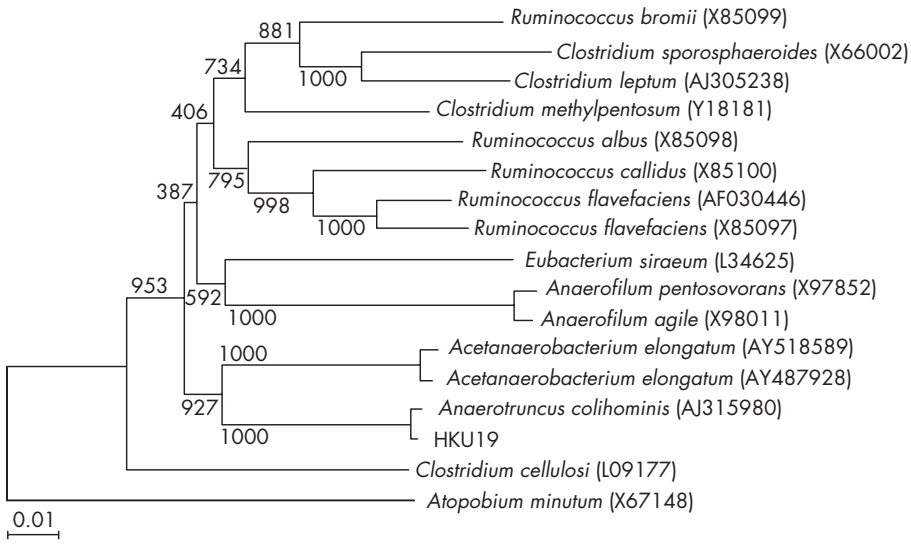

Figure 2 Phylogenetic tree showing the relationships of strain HKU19 to *Anaerotruncus colihominis* (Genbank accession number AJ315980) and members of other related genera. The tree was constructed by the neighbour-joining method and bootstrap values calculated from 1000 trees. A total of 1419 nucleotide positions were included in the analysis. The scale bar indicates the estimated number of substitutions per 100 bases using the Jukes–Cantor correction. Names and accession numbers are given as cited in the GenBank database.

Table 1 Comparison between strain HKU19 and *Anaerotruncus colihominis*[7]

Biochemical reactions/enzymes/ substrates	*A colihominis*	HKU19
Spore formation	−	+
Catalase	−	−
Aesculin hydrolysis	−	−
Gelatin hydrolysis	−	−
Arginine dehydrogenase	−	−
Alkaline phosphatase	−	−
Glutamic acid decarboxylase	−	−
Indole production	+	+
Phosphate choline		−
Urease	−	−
Reduction of nitrate	−	−
Reduction of triphenyl tetrazolium		−
Oxidation/fermentation of		
Arabinose	−	−
Cellobiose	−	−
Glucose	+	+
Glycerol		−
Lactose	−	−
Maltose	−	−
Mannitol	−	−
Mannose	+	+
Melezitose	−	−
Raffinose	−	−
Rhamnose	−	−
Salicin	−	−
Sorbitol	−	−
Sucrose		−
Trehalose		−
Xylose	−	−
α-Arabinosidase	−	−
α-Fucosidase	−	−
β-Fucosidase		−
α-Galactosidase	−	−
β-Galactosidase	−	−
β-Galactosidase-6-phosphate	−	−
α-Glucosidase	−	−
β-Glucosidase	−	+
β-Glucuronidase	−	−
α-Mannosidase	−	−
β-Lactosidase		−
β-Xylosidase		-
N-acetyl-glucosaminidase	−	−
Alanine arylamidase	−	−
Arginine arylamidase	−	−
Benzoyl-arginine arylamidase		−
Glutamyl glutamic acid arylamidase	−	−
Glycine arylamidase	−	−
Histidine arylamidase	−	−
Leucine arylamidase	−	−
Leucyl glycine arylamidase	−	−
Lysine arylamidase		+
Phenylalanine arylamidase	−	−
Proline arylamidase	−	−
Pyroglutamic acid arylamidase	−	−
Serine arylamidase	−	−
Tyrosine arylamidase	−	−
DNA G+C content (mol%)	54	53

C4, β-galactosidase-6-phosphate, glutamic acid decarboxylase, glycine arylamidase, histidine arylamidase, lipase C14, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, pyroglutamic acid arylamidase, serine arylamidase, tyrosine arylamidase and valine arylamidase activities are not detected. Cells are sensitive to vancomycin (5 μg) and kanamycin (1000 μg), but resistant to colistin sulphate (10 μg) identification discs. DNA G+C content is 53–54 mol%. The organism is isolated from human clinical specimens. The type strain is WAL 14 565T = CCUG 45 055T = CIP 107 765T.

ACKNOWLEDGEMENTS

This work was partly supported by the University Development Fund and University Research Grant Council Grant (HKU 7236/02M), The University of Hong Kong and the William Benter Infectious Disease Fund.

Authors' affiliations

S K P Lau, P C Y Woo, G K S Woo, A M Y Fung, A H Y Ngan, K Yuen, Department of Microbiology, The University of Hong Kong, Hong Kong
Y Song, C Liu, P Summanen, Research Service, VA Medical Center West Los Angeles, Los Angeles, USA
S M Finegold, Department of Microbiology, Immunology and Molecular Genetics, UCLA School of Medicine

Competing interests: None.

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