J Microbiol Immunol Infect.
2008;41:183-186

Case Report

Isolation of urease-positive *Ochrobactrum intermedium* in the stomach of a non-ulcer dyspeptic patient from north India

Mahesh S. Dharne[1], Sri Prakash Misra[2], Vatsala Misra[3], Manisha Dwivedi[2], Milind S. Patole[1], Yogesh S. Shouche[1]

[1]Molecular Biology Unit, National Centre for Cell Science, Pune; and Departments of [2]Gastroenterology and [3]Pathology, Moti Lal Nehru Medical College, Allahabad, India

Received: October 19, 2006 Revised: February 1, 2007 Accepted: March 27, 2007

Ochrobactrum intermedium is an opportunistic human pathogen found in immunocompromised individuals. We report the case of a north Indian patient with non-ulcer dyspepsia whose gastric biopsy revealed the presence of *O. intermedium*, along with *Helicobacter pylori*. Further description of *O. intermedium* was performed with 16S rRNA (1500 nucleotides) and *RecA* (1065 nucleotides) gene sequencing, and the identity and phylogenetic affiliation of the isolate was confirmed by 100% nucleotide similarity with *O. intermedium* LMG3301. Further investigation is required in order to evaluate the link between *H. pylori* and *O. intermedium* in the gastric niche.

Key words: *Helicobacter pylori*; *Ochrobactrum*; RecA recombinases; RNA, ribosomal, 16S

Introduction

The human stomach is colonized by diverse groups of bacteria, both pathogenic and non-pathogenic. Microorganisms in the stomach comprise a broad range of bacteria, including *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Fusobacteria*, streptococci and lactobacilli. Recently, it has been postulated that the stomach comprises 32 phylotypes from *Proteobacteria*, including 3 uncharacterized phylotypes [1]. *Helicobacter pylori*, a known causative agent of peptic ulcers and gastric cancer, colonizes the stomach of approximately 50% of the world's population. However, little is known regarding the presence of bacteria other than *H. pylori* in the human stomach.

We describe for the first time the presence of *Ochrobactrum intermedium* in the antrum of a non-ulcer dyspepsia patient from India, who was diagnosed positive for *H. pylori*.

The genus *Ochrobactrum* comprises saprophytic, Gram-negative, oxidase-producing, lactose-non-fermenting, urease-positive, capsulating, aerobic bacilli belonging to alpha 2 subgroup of the class *Proteobacteria* (previously described as *Achromobacter* spp. or CDC group Vd), with 6 different species at present [2]. *Ochrobactrum* is a close genetic relative of the genus *Brucella*, as evidenced by protein profiling, Western blot, 16S rRNA and *RecA* sequencing-based studies, immunoelectrophoresis and amplified fragment length polymorphism [2-4]. *Ochrobactrum anthropi* has been the causative agent of various clinical and nosocomial outbreaks [3].

O. intermedium is an emerging pathogen in liver abscess post-liver transplantation and in bladder cancer patients [5]. Clinical manifestations and diseases caused by *O. intermedium* are poorly known and there are no suitable techniques for discriminating organisms up to species level in clinical cases. Conventional methods such as biochemical characteristics and antibiotic profiling do not provide accurate identification [5].

Recently, it has been shown that recombinase gene subunit A (*RecA*) is a reliable marker for the discrimination of *Ochrobactrum* taxon at inter- and intraspecies level [2]. We report the isolation of *O. intermedium* from the stomach of a male patient with non-ulcer dyspepsia.

Corresponding author: Dr. Yogesh S. Shouche, Molecular Biology Unit, National Centre for Cell Science, Ganeshkhind Road, Pune University Campus, Pune 411 007, Maharashtra, India.
E-mail: yogesh@nccs.res.in

© 2008 Journal of Microbiology, Immunology and Infection

Case Report

A patient with dyspeptic symptoms of bloating, gaseousness and postprandial epigastric pain of five to eight months' duration underwent upper gastrointestinal endoscopy, as per the guidelines of the ethical committee at Motilal Nehru Medical College, India. The patient, designated as 'M86' in our study, was a 26-year-old male with non-ulcer dyspepsia. There was no history of medication except for occasional consumption of antacids. The patient denied taking non-steroidal anti-inflammatory drugs. He was vegetarian and did not consume alcohol. Clinical evaluation revealed mild epigastric tenderness. Ultrasound examination of the abdomen did not reveal any abnormality. The fasting and postprandial blood glucose, serum urea, bilirubin, amylase, transaminases and creatinine were within normal limits.

Upper gastrointestinal endoscopy did not reveal any abnormality. Antral biopsies were obtained and rapid urease test (HiMedia Laboratories, Mumbai, India) was positive. Hematoxylin and eosin-stained sections of antral biopsy showed focal areas of damage and mucin depletion of the epithelial lining. Gastric pits were mildly distorted and widely placed because of marked fibrosis, mainly in the upper part of the lamina propria. Gastric glands showed mild atrophy, mainly in the superficial areas. There was moderate infiltration by chronic inflammatory cells. Superficial mucosal capillaries were dilated and congested (Fig. 1). The organisms compatible with typical morphology of *H. pylori* were seen in the gastric pits. In addition, some Gram-negative short rods were visible. There was no evidence of neutrophil activity, intestinal metaplasia or lymphoid collection.

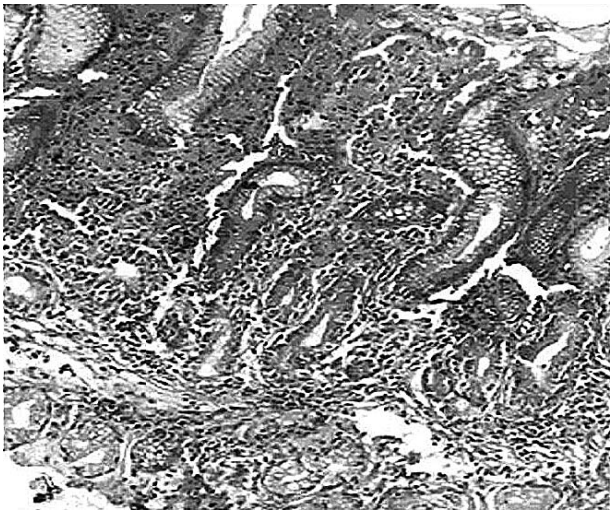

Fig. 1. Histopathological analysis of antral biopsy from the patient showing focal areas of damage and mucin depletion of the lining epithelium. The gastric pits are somewhat distorted and widely placed due to fibrosis mainly in the upper part of the lamina propria. Moderate infiltration by chronic inflammatory cells is also evident (hematoxylin and eosin stain, × 800).

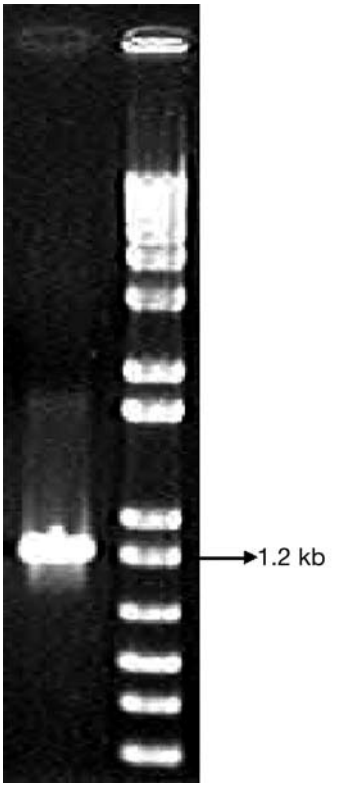

Fig. 2. Polymerase chain reaction (PCR) amplification for *Helicobacter pylori* detection. Lanes 1 and 2, PCR product amplified directly from the biopsy specimen using *H. pylori* specific 16S rDNA primers with varying DNA concentrations; lane 3, 1 kb plus DNA marker (Invitrogen, Carlsbad, USA).

Polymerase chain reaction from the total biopsy DNA with *H. pylori*-specific primers [6] confirmed the presence of *H. pylori* by sequence analysis (Fig. 2). However, by standard culture approach using *Brucella* chocolate agar (Difco, Detroit, MI, USA) supplemented with *H. pylori*-selective supplement (Dent, Oxoid, Hampshire, UK), there were no colonies with typical *H. pylori* morphology, but colonies with pigmentation and morphology different from *H. pylori* were successfully isolated and subcultured. The colonies that appeared after 48 h incubation were urease-positive, oxidase-positive and non-hemolytic. Biochemical analysis of the isolate with API 20NE (bioMérieux, Marcy l'Etoile, France) and conventional tests identified the isolate to be of genus *Ochrobactrum* [2]. 16S

© 2008 Journal of Microbiology, Immunology and Infection

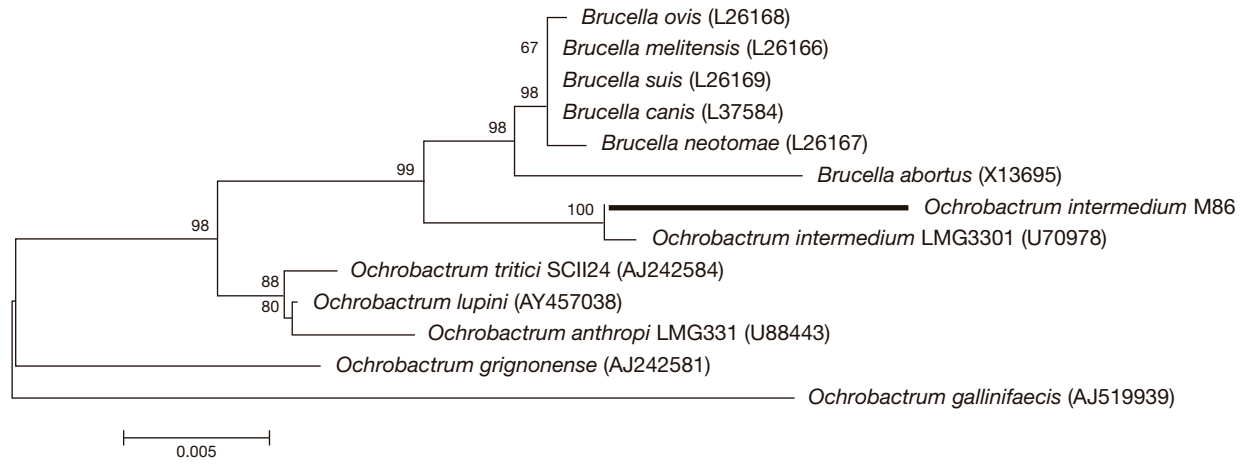

Fig. 3. Phylogenetic affiliation of the *Ochrobactrum intermedium* isolate: the neighbor-joining tree was prepared using the Kimura 2 model of Molecular Evolutionary Genetics Analysis (MEGA, Version 3.1) by aligning published sequences from Genbank of *Ochrobactrum intermedium* LMG 3301 (U70978), *Ochrobactrum anthropi* LMG 3331 (U88443), *Ochrobactrum grignonense* isolate OgA9aT, LMG 18954 (AJ242581), *Ochrobactrum tritici* isolate SCII24 (LMG 18957), *Ochrobactrum gallinifaecis* Iso 196T (DSM15295) and *Ochrobactrum lupini* LMG20667 (AY457038). For checking relatedness with genus *Brucella*, we included further sequences from GenBank *Brucella melitensis* ATCC 23456T (L26166), *Brucella ovis* American Type Culture Collection (ATCC) 25840 T (L26168), *Brucella suis* ATCC 23444T (L26169), *Brucella canis* ATCC 23365T (L37584), *Brucella neotomae* ATCC 23459T (L26167) and *Brucella abortus* (X13695).

rDNA gene sequence of the isolate revealed 99% homology with *O. intermedium* LMG 3301 in GenBank. In phylogenetic analysis using Molecular Evolutionary Genetics Analysis (MEGA, Version 3.1; Institute of Molecular Evolutionary Genetics, University Park, PA, USA) [7], the isolate clustered with *O. intermedium* LMG3301 (Fig. 3). The 16S rRNA sequence of the isolate M86 deposited to GenBank under the accession number DQ100421.

Susceptibility to various antimicrobials, including beta-lactams, cephalosporins, macrolides, quinolones, sulfonamides and aminoglycosides was determined on Mueller-Hinton agar by using standard commercial antibiotic octadiscs (HiMedia Laboratories). Zones of inhibition were calculated as per the recommendations of the National Committee for Clinical Laboratory Standards, 2002. The isolate was sensitive to tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tobramycin (10 µg), ciprofloxacin (5 µg) and ofloxacin (5 µg), and chloramphenicol (30 µg) and was resistant to 28 antibiotics, which included penicillin G (10 units), ampicillin (10 µg), cephalexin (30 µg), erythromycin (15 µg), gentamicin (10 µg), metronidazole (5 µg), carbenicillin (100 µg), trimethoprim (5 µg), norfloxacin (10 µg), imipenem (10 µg), kanamycin (30 µg), amikacin (30 µg) and amoxicillin-clavulanic acid (30 µg). The isolate was resistant to colistin (10 mg) and polymyxin B (300 units), indicating the authenticity and virulence of the species as *O. intermedium*.

Discussion

The human stomach harbors a diverse range of bacteria, ranging from non-pathogens to pathogens. However, the relationship between colonizing bacteria and clinical disease is frequently complex [8]. *Ochrobactrum* infection was first reported in the form of pancreatic abscess in 1980 [9]. *O. anthropi* and *O. intermedium* are well known opportunistic human pathogens causing bacteremia and sometimes serious diseases [3,10]. *O. anthropi* has been isolated from various clinical settings in immunocompetent and immunocompromised patients [11], including nosocomial sepsis in human immunodeficiency virus-positive patients [12], noma lesions, and septic shock and from infection complicating hemodialysis, vertebral osteomyelitis, urine, feces, wounds, throat and medical equipment. *O. intermedium* has been detected in post-liver transplant patients [13] and from clinical specimens [11]. Recently, *O. anthropi* was isolated from the stomach of squirrel monkeys (*Saimiri* sp.) which had developed gastritis [14].

We are not aware of any report in which *Ochrobactrum* spp. has been isolated from the stomach of human beings. A unique observation in the present

case report was evidence of severe fibrosis in the lamina propria of the gastric mucosa on histological examination of the gastric antral biopsy (Fig. 1). Whether or not this fibrosis was partially or totally due to *O. intermedium* is conjectural. In this context, it is interesting to note that *O. anthropi* has been shown to be associated with mild gastritis in squirrel monkeys. This case also had infection by *H. pylori*. Since the prevalence of *H. pylori* in the population is very high, whether or not it had a role in this patient's symptoms and histological findings is not clear [15]. Bacteria other than *H. pylori* have been found in cases of chronic active gastritis [16]. There are reports of gastritis due to enterococcci [17], and in some cases staphylococci have been shown to be associated with gastric disorders and isolated from antral biopsies [18] from patients with or without *H. pylori* colonization. Importantly, *H. pylori* and *O. intermedium* both produce urease, and thus the presence of *Ochrobactrum* may confound the urease test for *H. pylori*. The role of *Ochrobactrum* in gastric pathology remains uncertain and requires further investigation.

Acknowledgments

We are greatful to Padmashree Dr. G.C. Mishra for his constant support and encouragement to carry out the studies. The authors thank the Department of Biotechnology, New Delhi, India for providing financial support.

References

1. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci USA. 2006; 17:732-7.
2. Kämpfer P, Rosselló-Mora R, Scholz HC, Welinder-Olsson C, Falsen E, Busse HJ. Description of *Pseudochrobactrum* gen. nov., with the two species *Pseudochrobactrum asaccharolyticum* sp. nov. and *Pseudochrobactrum saccharolyticum* sp. nov. Int J Syst Evol Microbiol. 2006;56:1823-9.
3. Teyssier C, Marchandin H, Jean-Pierre H, Diego I, Darbas H, Jeannot JL, et al. Molecular and phenotypic features for identification of the opportunistic pathogens *Ochrobactrum* spp. J Med Microbiol. 2005;54:945-53.
4. Leal-Klevezas DS, Martinez-de-la-Vega O, Ramírez-Barba EJ, Osterman BJ, Martínez-Soriano P, Simpson J. Genotyping of *Ochrobactrum* spp. by AFLP analysis. J Bacteriol. 2005;187:2537-9.
5. Apisarnthanarak A, Kiratisin P, Mundy LM. Evaluation of *Ochrobactrum intermedium* bacteremia in a patient with bladder cancer. Diagn Microbiol Infect Dis. 2005;53:153-5.
6. Harper CM, Xu S, Feng Y, Dunn JL, Taylor NS, Dewhirst FE, et al. Identification of novel *Helicobacter* spp. from a beluga whale. Appl Environ Microbiol. 2002;68:2040-3.
7. Kumar SK, Tamura IB, Jakobsen IB, Nei M. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics. 2001;17:1244-5.
8. Lee SG, Kim C, Ha YC. Successful cultivation of a potentially pathogenic coccoid organism with trophism for gastric mucin. Infect Immun. 1997;65:49-54.
9. Appelbaum PC, Campbell DB. Pancreatic abscess associated with *Achromobacter* group Vd biovar 1. J Clin Microbiol. 1980;12:282-3.
10. Mastroianni A, Cancellieri C, Montini G. *Ochrobactrum anthropi* bacteremia: case report and review of the literature. Clin Microbiol Infect. 1999;5:570-3.
11. Xu J, Moore JE, Millar BC, Crowe M, McClurg R, Heaney LG. Identification of a novel alpha-proteobacterium causing bacteraemia in an immunocompetent patient. J Infect. 2003;47:167-9.
12. Manfredi R, Nanetti A, Ferri M, Chiodo F. Nosocomial sepsis due to *Ochrobactrum anthropi* in HIV positive patients: two case reports. Infez Med. 1999;7:119-24. [In Italian, English abstract].
13. Möller LV, Arends JP, Harmsen HJ, Talens A, Terpstra P, Slooff MJ. *Ochrobactrum intermedium* infection after liver transplantation. J Clin Microbiol. 1999;37:241-4.
14. Khanolkar-Gaitonde SS, Reubish GK Jr, Lee CK, Stadtländer CT. Isolation of bacteria other than *Helicobacter pylori* from stomachs of squirrel monkeys (*Saimiri* spp.) with gastritis. Dig Dis Sci. 2000;45:272-80.
15. Misra V, Misra SP, Dwivedi M, Singh PA. Point prevalence of peptic ulcer and gastric histology in healthy Indians with *Helicobacter pylori* infection. Am J Gastroenterol. 1997; 92:1487-91.
16. Heilmann KL, Borchard F. Gastritis due to spiral shaped bacteria other than *Helicobacter pylori*: clinical, histological, and ultrastructural findings. Gut. 1991;32:137-40.
17. El-Zimaity HM, Ramchatesingh J, Clarridge JE, Abudayyeh S, Osato MS, Graham DY. Enterococcus gastritis. Hum Pathol. 2003;34:944-5.
18. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. Am J Gastroenterol. 2006;101:1756-61.

© 2008 Journal of Microbiology, Immunology and Infection