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Surgical site abscess caused by *Lactobacillus fermentum* identified by 16S
ribosomal RNA gene sequencing

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Running title: *Lactobacillus fermentum* surgical site abscess

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Abstract

We report the first case of surgical site abscess caused by *Lactobacillus fermentum* from a 53-year old woman with squamous cell carcinoma of the esophagus after transthoracic esophagectomy and neoadjuvant chemoradiation. 16S rRNA gene sequencing is a useful tool to better characterize the epidemiology and clinical significance of *L. fermentum*.

Keywords: *Lactobacillus fermentum*, surgical site abscess, 16S rRNA gene sequencing

Text

Lactobacillus fermentum, one of the over 120 species in the genus *Lactobacillus*, was discovered in 1901 as a heterofermentative bacterium (Beijerinck, 1901). *L. fermentum* can be isolated from milk products, sourdough, fermenting plant material, manure and oral cavities and feces of human beings. The bacterium is most well known for its utilization as probiotic bacteria and it has been shown to be effective for the treatment of bacterial vaginosis and infantile and adult diarrhea (Gusils et al., 1999; Reid et al., 2003). *L. fermentum* is generally regarded as non-pathogenic. Only a few cases of *L. fermentum* infections have been reported (Colloc et al., 1978; Gallemore et al., 1995; Greig et al., 1998; Saxelin et al., 1996; Sriskandan et al., 1993). In this article, we describe the application of 16S rRNA gene sequencing, the *state-of-the-art* technology for classification and identification of anaerobic Gram-positive bacilli (Woo et al., 2002a, 2003; Lau et al., 2004a, 2004b), in characterizing a case of surgical site abscess caused by *L. fermentum*.

A 53-year-old Chinese woman was admitted because of erythema over the left neck wound 20 days after transthoracic esophagectomy. She had received the last session of neoadjuvant radiotherapy and chemotherapy for her supracarinal esophageal squamous cell carcinoma 13 weeks ago. Eight days post-esophagectomy, a seroma developed over the neck wound, which responded to drainage for 3 days. She was afebrile. Examination showed erythema and tenderness over the neck wound. Total white cell count was $6.2 \times 10^9/l$, hemoglobin level was 10.0 g/dl and platelet count was $418 \times 10^9/l$. Serum albumin was 36 g/l and globulin 40 g/l. Serum bilirubin, liver enzymes, urea and creatinine were within normal limits. Contrast computed tomography of the neck revealed

anastomotic leakage with a left paratracheal collection. Incision and drainage of the abscess was performed. Gram smear of the pus showed numerous leucocytes and Gram-positive bacilli. Culture of the pus recovered pure heavy growth of a Gram-positive, non-spore-forming bacillus. The abscess responded to drainage and 14 days of amoxicillin-clavulanate. Follow-up gastrograftin swallow did not reveal any more leakage and there was no relapse of the illness up to the time of writing, 60 days after discharge.

The pus isolate was a Gram-positive, non-spore-forming bacillus. It grew on sheep blood agar as non-hemolytic, gray colonies of 1 mm in diameter after 24 h of incubation at 37°C in ambient air. It also grew well on chocolate, MacConkey and Sabouraud (pH 5.6) agars and in 5% CO₂ and anaerobic environment. It was catalase negative and non-motile. Positive results were obtained for utilization of glucose, lactose, maltose, mannose, melibiose, raffinose, ribose, sucrose and trehalose. The Vitek system (ANI) identified the bacterium as 81% *Clostridium clostridioforme* and 15% *Clostridium butyricum* and the Phoenix Automated Microbiology System identified it as 99% *Arcanobacterium haemolyticum*. The minimal inhibitory concentration of vancomycin was >256 µg/ml.

Amplification and sequencing of the 16S rRNA gene (1481 bp) of the pus isolate, using primers LPW57 5'-AGTTTGATCCTGGCTCAG-3' and LPW205 5'-CTTGTTACGACTTCACCC-3' and according to a protocol we reported previously (Woo et al., 2002b), showed that there were only 2 (0.1%) base differences between the 16S rRNA gene sequence of the isolate and that of *L. fermentum* (GenBank accession no. AY929282) and 53 (3.8%) base differences between the 16S rRNA gene sequence of the

isolate and that of *L. gastricus* (GenBank accession no. AY253658), indicating that the isolate was a strain of *L. fermentum* (Fig. 1).

The present report represents the first case of *L. fermentum* surgical site infection reported in the literature. The clinical significance of the bacterium was evident by its isolation from abscess pus obtained during operation in pure heavy growth and the presence of abundant Gram-positive bacilli observed on direct Gram smear examination. Although growth on Sabouraud agar and vancomycin resistance suggested that it was a *Lactobacillus* species, two commercially available bacterial identification systems identified it as *Clostridium* species and *A. haemolyticum* respectively. Therefore, 16S rRNA gene sequencing was performed and showed that it was an isolate of *L. fermentum*, with only two bases difference between it and another strain of *L. fermentum* isolated from fermented cassava used for the preparation of Gari, a traditional African food (Kostinek et al., 2005). The poor performance of commercial kits in identifying *Lactobacillus* species is in line with the results of our recent study, which showed that all three *Lactobacillus* species recovered from blood cultures of our patients, *L. casei/paracasei*, *L. rhamnosus* and *L. salivarius*, were misidentified by three different commercial kits (Lau et al., 2006). In retrospect, the biochemical profile of the present isolate mostly fit that of *L. fermentum* described in Bergey's Manual of Systematic Bacteriology (Garrity, 2001), with the exception of galactose utilization.

Including the present case, only six cases of *L. fermentum* infections with clinical details available have been reported (Table 1) (Colloc et al., 1978; Gallemore et al., 1995; Greig et al., 1998; Saxelin et al., 1996; Sriskandan et al., 1993). Four were males and two

were females. The median age was 53.5 (range 16-71). All patients had major underlying diseases, with malignancies in three, post-liver transplant in one, haemophilia and AIDS in one and mitral valve insufficiency in one. The present case is the first one documented by 16S rRNA gene sequencing. The three patients in whom *L. fermentum* was recovered in pure culture survived, whereas in the other two in whom *L. fermentum* was concomitantly recovered with other bacteria and/or fungi, died.

Due to their abilities to survive in acidic environment, lactobacilli, including *L. fermentum*, have been found in the stomach of human beings (Daud Khaled et al., 1997). Furthermore, it has been demonstrated that *L. fermentum* is capable of inhibiting the adhesion or growth of various bacteria (Brashears et al., 2003; Miyamoto et al., 2000; Velraeds et al., 1996). We speculate that our patient, probably with *L. fermentum* as the predominant flora in the stomach as a result of the selective advantage of its survival in acidic environment and inhibition of growth of other bacteria, was leaked out through the poorly healed anastomotic site due to chemoradiation and caused abscess formation.

Nucleotide sequence accession number. The 16S rRNA gene sequence of the isolate has been deposited in GenBank (accession no. DQ779203).

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Legends to figures

Fig. 1. Phylogenetic tree showing the relationship of the pus isolate to the 20 most closely related *Lactobacillus* species. The tree was inferred from 16S rRNA data by the neighbor-joining method and rooted using the 16S rRNA gene sequence of *Staphylococcus aureus*. Bootstrap values were calculated from 1000 trees. 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
 Characteristics of patients with *Lactobacillus fermentum* infections reported in the literature with clinical details available

Characteristics	Patient no.					
	1	2	3	4	5	6
References	Colloc et al. 1978	Sriskandan et al. 1993	Gallemore et al. 1995	Saxelin et al. 1996	Greig et al. 1998	Present case
Geographical locations	France	Not mentioned	USA	Finland	United Kingdom	Hong Kong
Age/sex	M/54	M/46	F/16	M/55	M/71	F/53
Underlying diseases	Gastrectomy for cancer	Haemophilia, AIDS, neutropenia	Mitral valve insufficiency	Post-liver transplant	Malignant common bile duct obstruction, non-insulin dependent diabetes mellitus	Carcinoma of esophagus, post-chemotherapy, radiotherapy and esophagectomy
Community/hospital acquired	Not mentioned	Hospital acquired	Community acquired	Not mentioned	Community acquired	Hospital acquired
Clinical specimens positive for <i>L. fermentum</i>	Pleural fluid	Sputum	Blood culture	Blood culture	Ascitic fluid	Abscess pus
Methods for identification of <i>L. fermentum</i>	Not mentioned	Not mentioned, by reference laboratory	Pre-reduced, anaerobically sterilized (PRAS) biochemical system	API 50 CHL	API Strep and speciated by reference laboratory	16S rRNA gene sequencing
Other bacteria recovered in clinical specimens	<i>Candida tropicalis</i> , <i>Bacteroides distasonis</i>	<i>Candida albicans</i> , <i>Aspergillus fumigatus</i>	None	Not mentioned	None	None
Diagnosis	Purulent pleurisy	Pneumonia	Native mitral valve endocarditis	Pneumonia	Spontaneous bacterial peritonitis	Surgical site infection
Treatment	Not mentioned	Not mentioned	Penicillin G + gentamicin	Not mentioned	Drainage + penicillin G	Drainage + amoxicillin-clavulanate
Outcome	Died	Died	Remission	Not mentioned	Remission	Remission

