Abstract. – Objectives: Acute appendicitis is a common surgical emergency. The etiology and pathophysiology of appendicitis have been well investigated. *Aggregatibacter aphrophilus* is a fastidious gram-negative coccobacilli. Detection of this organism in clinical samples and its differentiation from *Haemophilus aphrophilus* or from *Aggregatibacter actinomycetemcomitans* in routine microbiology settings could be difficult.

Methods: In this rare case, we report the isolation of *Aggregatibacter aphrophilus* from the appendix of a 14-year old boy presented with acute appendicitis. The genotypic method using 16S rRNA sequencing was used for identification of the organism at species level.

Conclusion: This case highlights the importance of detecting fastidious and rare microorganisms such as *Aggregatibacter aphrophilus* that could be associated with acute appendicitis.

Key Words: *Aggregatibacter aphrophilus*, Acute appendicitis, *Haemophilus paraphrophilus*, 16S rDNA.

Case Report

A 14-year old boy with no previous medical illness presented to University of Malaya Medical Centre (UMMC) with right iliac fossa pain associated with diarrhoea and fever for four days. The pain was constant and was aggravated by movement. He was febrile (38.5°C) and his vital signs were stable. Physical examination revealed tenderness and guarding in the right iliac fossa. Laboratory evaluation included a complete blood count, which showed leucocytosis of 15,000/mm³ (79.7% neutrophils, 11% lymphocytes, and 3% eosinophils). The urinalysis was negative. Clinical diagnosis of acute appendicitis was made, and the patient was subjected to emergency appendicectomy with intravenous cefuroxime and metronidazole given as prophylaxis. Intra-operatively, the appendix was found to be grossly inflamed and swollen with some slough noted on the body of the appendix. There was about 15 ml of haemoserous fluid adjacent to it. Appendicectomy was performed without any complication. The resected specimen was sent for histological examination and a small piece of the inflamed tissue was also sent to the diagnostic microbiology laboratory. Post-operative recovery was uneventful and the patient was eventually discharged well. Histology of the resected specimen confirmed the diagnosis of acute appendicitis. The small piece of appendix tissue sent to the diagnostic microbiology laboratory, was cultured onto 5% sheep blood agar, MacConkey agar and chocolate agar plates. MacConkey agar plates were incubated in ambient air, 5% sheep blood agar and chocolate agar plates were incubated in 5% CO₂ at 35°C up to 48 hrs. The culture from the chocolate agar plate showed scanty growth of convex, opaque colonies after incubation in 5% CO₂ at 35°C for 48 hrs. The culture from the chocolate agar plate showed scanty growth of convex, opaque colonies after incubation in 5% CO₂ at 35°C for 48 hrs. Gram-stain smear showed small gram-negative cocco-bacilli. The isolate was tested for X and V factor dependent growth *in-vitro* and the result showed that it was X factor independent and V factor dependent. The factor V-dependency of this isolate ruled out *H. aphrophilus* which is factor V- and X-independent. The oxidase test was positive. This isolate was identified as *Haemophilus paraphrophilus/Haemophilus aphrophilus* (99.3%, very good identification) by API-NH 7162 pro-
file (BioMerieux, Marcy-l’Etoile, France). The API report suggested testing for V factor dependence, which was positive, indicating it was *Haemophilus paraphrophilus*. Biochemical reactions indicated that onitrophenyl-β-D-galactosidase (ONPG) test was positive, and catalase test was negative. These results ruled out *Aggregatibacter actinomycetemcomitans*. The indole, urease test were negative. In carbohydrate utilization tests the organism could ferment glucose and lactose. API result showed that the organism was positive for D-glucose, fructose, maltose, saccharose (sucrose), 4-nitrophenyl-phosphate2CHA (PAL), 4-nitrophenyl-β-D-galactopyranoside (βGAL), ϒ-glutamyl-4-methoxy-β-naphthylamide (GGT). The disk diffusion method of antibiotic sensitivity testing was performed according to Clinical Laboratory Standards Institute (CLSI) guideline (2009)1. The interpretation of results was done following the criteria for *Haemophilus species*. The isolate was sensitive to ampicillin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, cefuroxime and ceftriaxone.

Recently the complete genome sequence of *Aggregatibacter (Haemophilus) aphrophilus* strain NJ8700 has been assigned2. For further confirmation of this isolate, molecular identification using PCR amplification and sequencing of 16S rDNA (ABI3730 XL DNA Analyser, Applied Biosystems, Renton, WA, USA)3 were performed. Sequence results from each amplified fragment were aligned and assembled into contigs to obtain a complete 16S rDNA consensus sequence using Sequencher™ ver. 4.0.5 (Gene Codes Corporation, Ann Arbor, MI, USA) which confirmed identification as *Aggregatibacter aphrophilus*.

**Discussion**

Appendicitis is the leading cause of acute abdominal pain requiring surgical procedure. Post-operative complications including wound infection and intra-abdominal abscess are responsible for a significant morbidity of appendicitis in spite of utilizing improved surgical techniques4. The exact underlying mechanisms of acute appendicitis are still obscure but likely due to luminal obstruction by lymphoid hyperplasia, fecal material and appendicoliths which play a key pathogenic role. Clinical manifestations are due to the ischemic injury that follows bacterial proliferation and additional inflammatory exudates and edema4-6. The bacteria most often associated with acute appendicitis include *Escherichia coli*, *Bacteroides melaninogenicus*, *Bacteroides fragilis* group, *Provetella* spp., *Bilophila wadsworthia*, *Peptostreptococcus* spp., *Enterobacteriaceae* and viridans streptococci (particularly *Streptococcus anginosus* group)7-9. *Haemophilus aphrophilus* and *Haemophilus paraphrophilus* are fastidious gram-negative coccobacilli belonging to the HACEK group (*Haemophilus* spp., *Actinobacillus* actinomycetemcomitans, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* spp.). Both these organism are part of the normal flora of the human oral cavity and pharynx and are now reclassified as *Aggregatibacter aphrophilus*10-11. This bacterium may be responsible for infective endocarditis, paronychia, brain and cerebral abscesses, osteomyelitis of the jaw and recently reported as a rare pathogen associated with spondylodiscitis12-13.

Norskov-Lauritsen and Kilian14 proposed *Aggregatibacter* gen. nov. as the generic name for the group *Aggregatibacter*. This generic name for the group was proposed due to conspicuous growth of the bacteria in broth as small granules adhering to the walls of the test tube. The association of *Haemophilus paraphrophilus* with acute appendicitis has been documented as early as 1981 by Kilian and Frederiksen15 while five cases of *Aggregatibacter segnis* (formerly known as *Haemophilus segnis*) appendicitis have been reported previously16. The paucity of documentation of this organism could be due to two reasons. Firstly microbiological investigation for acute appendicitis is not a common practice and, therefore, the clinical sample for microbiological examination may not be sent to microbiology laboratory. Secondly, presence of this organism in clinical samples remains undetected unless appropriate microbiological method is used. Identification of *Haemophilus paraphrophilus* and its differentiation from *Haemophilus aphrophilus* or from *Aggregatibacter actinomycetemcomitans* in routine microbiology settings could be difficult, due to the similarities in colonial morphology and growth requirements. Isolation of normal flora like *Aggregatibacter aphrophilus* from rare cases such as spondylodiscitis recently can highlight the association of this organism with infections. This organism can be further studied to identify its pathogenic role and its virulence in relation to infections caused in humans.
In conclusion, in this brief report, we identified *Aggregatibacter aphrophilus* which was associated with acute appendicitis in a patient. Identification of *Aggregatibacter aphrophilus* in the microbiology laboratory and its association with infections in patient could highlight this organism as a potential human pathogen.

Acknowledgements

The 16S rDNA gene sequencing was funded by a University of Malaya F vote grant (FS 243/2008 B).

References


