Methanobrevibacter smithii tonsillar phlegmon: a case report

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Abstract

Untreated tonsillar phlegmon is a life-threatening condition commonly caused by Streptococcus pyogenes and Fusobacterium necrophorum, among other pathogens. Here, using specific laboratory tools, we detected Methanobrevibacter smithii in addition to S. pyogenes. This unprecedented observation questions the role of methanogens in phlegmon and the optimal treatment of this mixed infection.

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Introduction

Tonsillar phlegmon, a retropharyngeal abscess resulting from the infection of the peritonsillar space and the pharyngeal constrictor muscle through the connective tissue of the amygda [1], is a life-threatening situation in the case of cellulitis, with extension to the mediastinum and potential extensive venous thrombosis up to the cavernous sinus [2]. Tonsillar phlegmon is affecting particularly adolescent and young adults [3], complicating acute pharyngitis, recurrent pharyngitis, and chronic tonsillitis [4].

Current microbiological documentation, mostly based on direct microscopic observation and culture of pus specimens, yields a polymicrobial infection of aerobic and anaerobic bacteria, dominated by Streptococcus pyogenes (group A Streptococcus), the only established pathogen in tonsillar abscess [5–7]. Also, streptococci (S. pyogenes, Streptococcus milleri, Streptococcus viridans), Haemophilus spp., and anaerobes including Fusobacterium necrophorum, Fusobacterium nucleatum, Prevotella melaninogenica, Prevotella intermedia, and Peptostreptococcus spp. are isolated from most patients (82–90%) [2,8,9].

In this situation of bacterial tonsillar phlegmon, detection of methanogenic archaea, mainly represented by Methanobrevibacter smithii, the most abundant methanogenic archaea species in the human gut [10] and which are recently emerging as a copathogen in various disease situations [11–14], would require specific laboratory methods.

Accordingly, here, we have used such specific methods we are mastering to explore the presence of methanogens in one case of tonsillar phlegmon.

Case presentation

A 22-year-old man was admitted to the emergency unit for a two-day progressively worsening dysphagia with odynophagia and bilateral pharyngeal pain, associated with sweating without fever. The patient self-treated himself with paracetamol without antibiotics or nonsteroidal anti-inflammatory drugs. Clinical examination revealed bilateral inflammatory oropharynx and trismus, no sign of cellulitis, and no palpated cervical lymphadenopathy. Nasofibroscopy disclosed no lesion in the oropharynx with mobile and free vocal cords. Remarkable lab-
Laboratory test values included leucocytosis at a level of 17 G/L with neutrophils at a level of 12 G/L and a C-reactive protein level at 132 mg/L. A cervical computed tomography scan showed bilateral tonsillar hypertrophy with a left tonsillar phlegmon without extension to the prevertebral space and without anomalies on the internal jugular venous and bilateral cervical adenopathies (Fig. 1). Intravenous amoxicillin and clavulanic acid at a dose of 1 g three times a day and 500 mg of metronidazole three times a day were started 24 hours before the patient benefited from surgical drainage of the left phlegmon. Per-operative papillomatosis was observed on the left tonsil, as confirmed by pathology that disclosed inflammatory papilloma without dysplasia. The pus collected from the phlegmon was immediately inoculated into one aerobic and one anaerobic blood culture bottle (Virtuo; bioMérieux, La Balme-les-Grottes, France) and incubated in the BACT/ALERT® VIRTUO® system (bioMérieux). Growth was detected in aerobic and anaerobic bottles after 3.6 and 4.3-hour incubation, respectively, and direct microscopic examination yielded gram-positive cocci. Broth culture was inoculated on Chocolate agar PolyViteX medium (bioMérieux) and COLUMBIA ANC medium (bioMérieux); Chocolate agar was incubated at 37°C and 5% CO₂ for 5 days to grow aerobic bacteria, and 5% sheep blood Columbia agar medium (bioMérieux) was incubated under strict anaerobiosis conditions at 37°C for 10 days to grow anaerobic bacteria. After 24-hour incubation, colonies grown on Columbia and Chocolate agar PolyViteX media were then identified as Streptococcus pyogenes using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as previously described by Seng et al [15]. Antibiotic susceptibility testing was carried out on the isolated strain as previously described by Haldorsen et al [16] and indicated wild-type S. pyogenes susceptible to amoxicillin, erythromycin, vancomycin, teicoplanin, clindamycin, and pristinamycin and exhibiting a low-level susceptibility to gentamicin. The final antibiotic therapy was amoxicillin and clavulanic acid at a dose of 1 gr three times a day and 500 mg of metronidazole three times a day for 10 days. The patient was cured after 15 days of follow-up.

For molecular detection of methanogens, DNA was extracted from the pus sample using the automated extractor EZ1 advanced XL with the EZ1 DNA Tissue kit (Qiagen, Courtaboeuf, France) after 20-minute sonication. Amplification of the archaeal 16S rRNA gene was performed as previously described by Drancourt et al [14]. Sequencing the amplicon yielded 100% sequence similarity with the reference M. smithii ATCC 35061 homologous sequence (GenBank accession number: NC_009515).

Furthermore, to determine whether the pus sample was monomicrobial or polymicrobial, we completed routine bacteriological investigations by using the rapid culturomic protocol as previously described by Naud et al [17]. Seven cultured bacterial species including Morganella morganii, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Enterococcus faecalis, and Bacteroides vulgatus were finally identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [15]. However, tentative isolation of M. smithii failed.

**Discussion**

We are reporting the unprecedented detection of the methanogen *M. smithii* in the pus sample collected from a case of tonsillar phlegmon. While this detection was ascertained by the negativity of the negative controls and the fact that the amplicon was sequenced, the very same pus also yielded a total of eight different bacterial species, which is in agreement with previous knowledge regarding the bacterial species reported to be associated with tonsillar phlegmon [18–20].

Until now, the presence of methanogens has never been reported in tonsil infections; this is due to the fact that methanogens are not systematically searched and that their culture remains fastidious. Here, the reason for no growth of *M. smithii* may be attributed to the fact that methanogens are extremely sensitive to oxygen and to the fact that phlegmon pus was collected after the patient received metronidazole, one of the few antibiotics shown to be effective *in vitro* against methanogens [21].
Although previously unreported, here, detection of *M. smithii* in tonsillar pus was not surprising as *M. smithii* is known to colonize the oral fluid microbiota [22], being further implicated in oral cavity dysbiosis such as periodontitis and peri-implantitis [23,24]. The presence of methanogens in saliva reinforces the hypothesis of a possible inflammation of the salivary glands that would cause tonsillar phlegmon [2,25].

In this patient, *M. smithii* was detected in a pus sample also containing eight different bacterial species, and such an association of *M. smithii* with bacteria, chiefly anaerobes, is constant in all the previous descriptions of *M. smithii* coinfections [11–14]. These bacteria essentially belong to the enterobacterial and bacteroidal orders comprising hydrogen producers [26,27]. Here, we suggest the possible involvement of *M. smithii* in this mixed infection by promoting the growth of aerobic and anaerobic bacteria via syntrophic interactions, which are the real pathogens. The exact mechanism is still poorly understood, but it would seem that methanogens control the production of essential growth factors for bacteria, such as short-chain fatty acids, by continuously maintaining a low partial pressure of H₂ [28,29] and may also play occasional roles in the development of tonsillar phlegmon and spread of infection.

In conclusion, this case report indicates that tonsillar phlegmon should no longer be regarded as a bacterial infection, but rather a mixed infection comprising anaerobes with methanogens. When the relative role of these different microorganisms should be reevaluated as methanogens are producing methane, which leads to tissue and cell damage [30,31] and is potentially responsible for gas radiologically visible in some cases of tonsillar phlegmon [32,33], it further questions the importance of prescribing antibiotics active against methanogens, such as in this patient in whom medical evolution was favourable.

**Transparency declaration**

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**Ethical approval**

Ethical approval was obtained from the IHU Méditerranée Infection Ethic Committee (no. 2021-010).

**Author contributions**

D.K. performed the experiments, data interpretation, and manuscript drafting; G.F. contributed to study design and manuscript drafting; M.J. and R.T. contributed to sample collection and data interpretation; D.M. contributed to study design and implementation, data analysis, result interpretation, and manuscript writing; G.G. contributed to study design and implementation, data analysis, and manuscript writing. All authors have read and agreed to the published version of the manuscript.

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**References**

[1] Klug TE. Peritonsillar abscess: clinical aspects of microbiology, risk factors, and the association with parapharyngeal abscess. Dan Med J 2017 Mar;64(3):B333. PMID: 28260599.

[2] Klug TE, Greve T, Henzle M. Complications of peritonsillar abscess. Ann Clin Microbiol Antimicrob 2020 Jul 30;19(1):32.

[3] Risberg S, Engfeldt P, Hugosson S. Incidence of peritonsillar abscess and relationship to age and gender: retrospective study. Scand J Infect Dis 2008;40(10):792–6.

[4] Powell J, Wilson JA. An evidence-based review of peritonsillar abscess. Clin Otolaryngol 2012 Apr;37(2):136–45.

[5] Galioto NJ. Peritonsillar abscess. Am Fam Phys 2008 Jan 15;77(2):199–202. PMID: 18246890.

[6] Brook I. The role of anaerobic bacteria in tonsillitis. Int J Pediatr Otorhinolaryngol 2005 Jan;69(1):9–19.

[7] Klug TE, Henriksen JJ, Fuursted K, Ovesen T. Significant pathogens in peritonsillar abscesses. Eur J Clin Microbiol Infect Dis 2011 May;30(5):619–27.

[8] Jousimies-Somer H, Savolainen S, Mäkitie A, Ylikoski J. Bacteriologic findings in peritonsillar abscesses in young adults. Clin Infect Dis 1993 Jun;16(Suppl. 4):S292–8.

[9] Brook I, Frazier EH, Thompson DH. Aerobic and anaerobic microbiology of peritonsillar abscess. Laryngoscope 1991 Mar;101(3):289–92.

[10] Dridi B, Henry M, El Khéchine A, Raout D, Drancourt M. High prevalence of Methanobrevibacter smithii and Methanosphaera stadtmanae detected in the human gut using an improved DNA detection protocol. PLoS One 2009 Sep 17;4(9):e7063.

[11] Nkamga VD, Lotte R, Roger PM, Drancourt M, Ruimy R. Methanobrevibacter smithii and Bacteroides thetaiotaomicron cultivated from a chronic paravertebral muscle abscess. Clin Microbiol Infect 2016 Dec;22(12):1008–9.

[12] Grine G, Lotte R, Chirio D, Chevalier A, Raoul D, Drancourt M, et al. Co-culture of Methanobrevibacter smithii with enterobacteria during urinary infection. EBioMedicine 2019 May;43:333–7.
[13] Sogodogo E, Fellag M, Loukil A, Nakanga VD, Michel J, Dessi P, et al. Nine cases of methanogenic archaea in Refractory sinusitis, an emerging clinical Entity. Front Publ Health 2019 Mar 4;7:38.

[14] Drancourt M, Djemai K, Gouriet F, Grine G, Loukil A, Bedotto M, et al. Methanobrevibacter smithii archaemia in febrile patients with bacteremia, including those with endocarditis. Clin Infect Dis 2020 Jul 15;cia998.

[15] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identifi

cation of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009 Aug 15;49(4):543–51.

[16] Haldorsen B, Giske CG, Hansen DS, Helgason KO, Kahlmeter G, Löhr IH, et al., NordicAST CPE Study Group. Performance of the EUCAST disc diffusion method and two MIC methods in detection of Enterobacteriaceae with reduced susceptibility to meropenem: the NordicAST CPE study. J Antimicrob Chemother 2018 Oct 1;73(10):2738–47.

[17] Naud S, Khelfa S, Mbogning Fonkou MD, Dione N, Lagier JC, Raoult D. Proof of concept of culturomics use of time of care. Front Cell Infect Microbiol 2020 Nov 23;10:524769.

[18] Gavriel H, Lazarovitch T, Pomortsev A, Eviatar E. Variations in the microbiology of peritonsillar abscess. Eur J Clin Microbiol Infect Dis 2009 Jan;28(1):27–31.

[19] Johnston J, Stretton M, Mahadevan M, Douglas RG. Peritonsillar abscess: a retrospective case series of 1773 patients. Clin Otolaryngol 2018 Jun;43(3):940–4.

[20] Slouka D, Hanakovs J, Kostlivy T, Skopek P, Kubec V, Babuska V, et al. Epidemiological and microbiological aspects of the peritonsillar abscess. Int J Environ Res Publ Health 2020 Jun 5;17(11):4020.

[21] Khelfa S, Brunel JM, Raoult D, Drancourt M. Hydrophobicity of imidazole derivatives correlates with improved activity against human methanogenic archaea. Int J Antimicrob Agents 2013 Jun;41(6):544–7.

[22] Grine G, Terrer E, Boualam MA, Aboudharam G, Chaudet H, Ruimy R, et al. Tobacco-smoking-related prevalence of methanogens in the oral fluid microbiota. Sci Rep 2018 Jun 15;8(1):9197.

[23] Efenberger M, Agier J, Pawłowska E, Brzeziińska-Blaszczuk E. Archaea prevalence in inflamed pulp tissues. Cent Eur J Immunol 2015;40(2):194–200.

[24] Sogodogo E, Doumbo O, Aboudharam G, Kouriba B, Diawara O, Koita H, et al. First characterization of methanogens in oral cavity in Malian patients with oral cavity pathologies. BMC Oral Health 2019 Oct 30;19(1):232.

[25] Passy V. Pathogenesis of peritonsillar abscess. Laryngoscope 1994;104:185–90.

[26] Suzuki A, Ito M, Hamaguchi T, Mori H, Takeda Y, Baba R, et al. Quantification of hydrogen production by intestinal bacteria that are specifically dysregulated in Parkinson’s disease. PloS One 2018 Dec 26;13(12): e0208313.

[27] Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. Hydrogen cross-feeders of the human gastrointestinal tract. Gut Microbiol 2019;10(3):270–88.

[28] Samuel BS, Gordon JJ. A humanized gnotobiotic mouse model of host-archael-bacterial mutualism. Proc Natl Acad Sci U S A 2006 Jun 27;103(26):10011–6.

[29] Conway de Macario E, Macario AJ. Methanogenic archaea in health and disease: a novel paradigm of microbial pathogenesis. Int J Med Microbiol 2009 Feb;299(2):99–108.

[30] Roccanina D, Lauritano EC, Gabrielli M, Franceschi F, Ojetti V, Gasbarrini A. The role of methane in intestinal diseases. Am J Gastroenterol 2010 Jun;105(6):1250–6.

[31] Piqué JM, Pallarés M, Cusó E, Vilarr-Bonet J, Gassull MA. Methane production and colon cancer. Gastroenterology 1984 Sep;87(3):601–5.

[32] Bamgbose BO, Raprecht A, Hellstein J, Timmons S, Qian F. The prevalence of tonsilloliths and other soft tissue calcifications in patients attending oral and maxillofacial radiology clinic of the university of Iowa. ISRN Dent 2014 Jan 22:2014;839635.

[33] Cappa EF, Kinsella JJ, Gupta M, Bhatki M, Opatowsky MJ. Emergency imaging assessment of acute, nontraumatic conditions of the head and neck. Radiographics 2010 Sep;30(5):1335–52.