



# Methanobrevibacter smithii tonsillar phlegmon: a case report

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Revised version

1 ***Methanobrevibacter smithii* tonsillar phlegmon: A case report.**

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20 **ABSTRACT**

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

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27 **Keywords:** *Methanobrevibacter smithii*, methanogens, tonsillar phlegmon, *Streptococcus*  
28 *pyogenes*.

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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 amygdala [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 specimen, 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% to 90%) [2, 8, 9].

In this situation of bacterial tonsillar phlegmon, detection of methanogenic archaea, mainly represented by *Methanobrevibacter smithii* (*M. smithii*), the most abundant methanogenic archaea species in the human gut [10] and recently emerging as co-pathogen in various disease situations [11-14] would requires 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 with paracetamol without antibiotic nor non-steroidal anti-inflammatory drugs. Clinical examination revealed bilateral

inflammatory oropharynx and trismus, no sign of cellulitis, nor palpated cervical lymphadenopathy. Nasofibroscope disclosed no lesion in the oropharynx with mobile and free vocal cords. Remarkable laboratory test values included leukocytosis at 17 G/L with neutrophilic cells 12G/L and C-reactive protein at 132 mg/L. A cervical CT 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 (Figure 1). Intravenous amoxicillin and clavulanic acid 1gr three times a day and metronidazole 500 mg three times a day was started 24 hours before the patient benefited from a surgical drainage of the left phlegmon. Per-operative papillomatosis was observed on the left tonsil, as confirmed by pathology which disclosed inflammatory papilloma without dysplasia. The pus collected from the phlegmon was immediately inoculated into one aerobic and one anaerobic blood culture bottles (Virtuo; bioMérieux, La Balme les Grottes, France), incubated in the BACT/ALERT® VIRTUO® automaton (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), incubated at 37°C under 5% CO<sub>2</sub> for 5 days to grow aerobic bacteria; and 5% sheep blood Columbia agar medium (bioMérieux) incubated under strict anaerobiosis at 37°C for 10 days to grow anaerobic bacteria. After 24-hour incubation, colonies grown on Columbia and Chocolat PolyVitex media, and then identified as *Streptococcus pyogenes* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) as previously described [15]. Antibiotic susceptibility testing was done on the isolated strain as previously described [16] and indicated a wild-type *S. pyogenes* susceptible to amoxicillin, erythromycin, vancomycin, teicoplanin, clindamycin and pristinamycin; and exhibiting a low-level susceptibility to gentamicin. The final antibiotherapy was amoxicillin

and clavulanic acid 1gr three times a day and metronidazole 500 mg 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 automat extractor EZ1 advanced XL with EZ1 DNA Tissue kit (Qiagen, Courtaboeuf, France) after 20-minute sonication. Amplification of the archaeal 16S rRNA gene was performed as previously described [14]. Sequencing the amplicon yielded 100 % sequence similarity with the reference *M. smithii* ATCC 35061 homologous sequence (GenBank accession number NC\_009515).

Further, to determine whether the pus sample was monomicrobial or polymicrobial, we completed the routine bacteriological investigations by using the rapid culturomic protocol as previously described [17]. Seven cultured bacterial species including *Morganella morganii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Escherichia coli*, *Enterococcus faecalis* and *Bacteroides vulgatus*, were finally identified by MALDI-TOF-MS [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 tonsillar phlegmon. While this detection was ascertained by the negativity of the negative controls and the fact that amplicon was sequenced, the very same pus also yielded a total of eight different bacterial species, 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 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 few antibiotics shown to be *in vitro* effective against methanogens [21].

Although previously unreported, here detection of *M. smithii* in a 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 of 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 H<sub>2</sub> [28, 29] and may also play occasional roles in the development of tonsillar phlegmon as well as spread of infection.

In conclusion, this case report indicates that tonsillar phlegmon should no longer be regarded as a bacterial infection, but rather an infection mixing anaerobes with methanogens. When the relative role of these different microorganisms should be reevaluated as methanogens are producing methane which is a tissue and cell damage [30, 31], potentially responsible for gas radiologically visible in some cases of tonsillar phlegmon [32, 33], it

127 further questions the importance of prescribing antibiotics active against methanogens, such  
128 as in this patient which medical evolution was favorable.

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**Conflict of interest:**

The authors declare no conflicts of interest.

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**Author Contributions:** D.K; performed the experiments; data interpretation; manuscript drafting; G.F; study design and manuscript drafting; M.J and R.T; sample collection and data interpretation; D.M; study design and implementation, data analysis, results interpretation, and manuscript writing; G.G; 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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**Figure 1. head and neck CT-scan sagittal plan showing the tonsillar phlegmon (arrow) in which PCR detected *Methanobrevibacter smithii*, in addition to cultured bacteria.**

