Methanobrevibacter smithii tonsillar phlegmon: a case report

K. Djemai[1,2], F. Gouriet[1,2], J. Michel[3], T. Radulesco[3], M. Drancourt[1,2] and G. Grine[2,4]

1) *Aix-Marseille Univ, IRD, MEPHI,* 2) *IHU Méditerranée Infection,* 3) *CHU Hôpital de la Conception, Service ORL et Chirurgie cervico-faciale and* 4) *Aix-Marseille Université, UFR Odontologie, Marseille, France*

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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Corresponding author: G. Grine, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005, Marseille, France
E-mail: grineghiles@gmail.com

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 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-

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oratory 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% CO_2 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 antibiotherapy 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

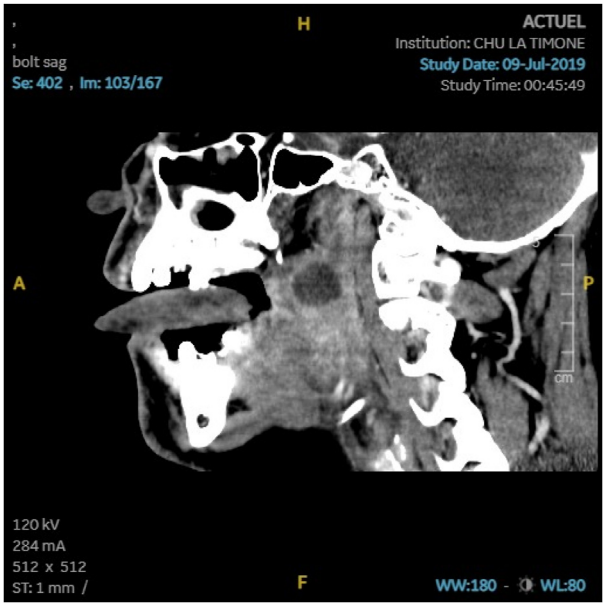

FIG. 1. Head and neck CT scan: sagittal plane showing the tonsillar phlegmon (arrow), in which PCR detected *Methanobrevibacter smithii*, in addition to cultured bacteria. CT, computed tomography; PCR, polymerase chain reaction.

previously described by Naud et al [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 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].

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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 H_2 [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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