

Peer Review File

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Reviewer A:

This is a well-written case report of a case of pyogenic thigh abscess caused by *Strep constellatus* in a patient with bronchiectasis.

The authors describe the clinical presentation and discuss the potential mechanisms of pathogenesis of the thigh abscess with the causative organism of *Strep constellatus*.

Major Comment 1: The authors state that the patient presented with an exacerbation of bronchiectasis and make reference to the respiratory symptoms but there is no description of the respiratory symptoms. The clinical picture presented is more in keeping of a patient presenting with an abscess than an exacerbation of bronchiectasis. The presence of rigors, persistent fever, reduced albumin, pronounced neutrophilia and absence of any consolidation on thoracic CT imaging make it much more likely that the thigh abscess was the primary, if not the sole cause for the presentation. CT imaging actually demonstrates plugging of bronchiectatic airways in the left lower lobe.

Please provide details of the respiratory symptoms and respond to the comments above.

Reply 1: Thank you for your comment. Firstly, we have provided details of the respiratory symptoms (see Page 4, line 76-77). Secondly, we agree the reviewer's comment that the thigh abscess may be the primary cause for the infectious symptoms, such as rigors, persistent fever and pronounced neutrophilia, but his change in cough frequency and increase in purulent sputum are the common features of bronchiectasis exacerbation.

Changes in the text: A 64-year-old Chinese man, who was a farmer and had previously been diagnosed with bronchiectasis in a local hospital based on chronic cough and expectoration (lasting >10 years), was admitted to our hospital after experiencing exacerbation involving nine days of rigor, moderate fever, increased cough and expectoration of white-yellow purulent sputum, as well as four days of pain in the right thigh (Case presentation, paragraph 1).

Major Comment 2: The authors state that the oral cavity is the main source of *S. constellatus* but they do not include any description of the oral health of this patient, which is of critical importance in this study, particularly since this is more likely to

have been the primary source of infection with haematogenous spread to the thigh as the cause of the abscess. I am not convinced that this patient had evidence of an exacerbation of bronchiectasis or that the alleged exacerbation was a cause of immunocompromised leading to development of a thigh abscess.

Please provide details of the patient's dental and oral examination, including radiographs. Please provide evidence to justify the contention that this patient was immunocompromised and that this was as a consequence of an exacerbation of bronchiectasis. Please provide evidence that *S. constellatus* is a pathogen in exacerbation of bronchiectasis in this patient (and / or in other patients).

Reply 2: Thank you for your comment. Firstly, we have provided details of the dental and oral examination, but the patient did not finish the radiograph of oral (**see Page 5, line 97-98**). Secondly, it seems that poor oral hygiene was the more likely cause of bacteremia due to *S. constellatus*, and we also are uncertain that *S. constellatus* is a pathogen in exacerbation of bronchiectasis in our patient. However, based on supplementary previous studies, we think the exacerbation of bronchiectasis may be another predisposing factor of *S. constellatus* infection and the *S. constellatus* should still be considered a potential pathogen to exacerbation of bronchiectasis, but these presumptions need further confirmation (**see Page 9-10, line 191-211**).

Changes in the text: Although infections caused by *S. constellatus* can be observed in individuals with normal immunity, a growing list of conditions were identified as potential predisposing factors, such as alcohol abuse, trauma, surgery, immunosuppression, multimorbidity, rheumatic diseases, cardiovascular diseases, chronic obstructive pulmonary disease, concurrent infection, and diabetes mellitus (3,18). Our patient had chronic underlying bronchiectasis and was suffering an acute exacerbation, so further work should examine whether this condition also is a predisposing factor of *S. constellatus* infection. In addition, the origin of the *S. constellatus* in the thigh abscess in our patient is unclear. It seems that poor oral hygiene was the most likely cause of bacteremia due to *S. constellatus*. However, the other possibility is the lung, even if sputum culture tests were negative, because primary lung infection can lead to pyopneumothorax and pulmonary abscess (7,19,20). Indeed, this can further evidence by another study which showed the *S. anginosus* group can induce chronic pulmonary infections and was the numerically dominant pathogen at the onset of 39% of acute pulmonary exacerbations in cystic fibrosis patients (21). Those investigators recommended expanding *S. anginosus* group surveillance to bronchiectasis patients. Thus, we believe that *S. constellatus*, although it is uncommon, should still be considered as a potential pathogen to exacerbation of bronchiectasis, especially in patients with secondary abscesses in other tissues and organs. To further our understanding of *S. constellatus*, the link between it and bronchiectasis should be explored in future work (**Discussion,**

paragraph 5).

Major Comment 3: The history is incomplete and missing key pieces of information that are relevant to this case.

Please provide further details about this patient's medical history, including details of previous bronchiectasis-associated infections (and microbiology), bronchiectasis causation, medication and, importantly, occupational and social history.

Please state whether there was any recent history of trauma, even if minor.

Reply 3: Thank you for your comment. Firstly, we have supplemented some details about this patient's medical history, including details of previous bronchiectasis-associated infections (and microbiology), bronchiectasis causation, medication and, importantly, occupational and social history (see Page 4-5, line 73-91). In addition, we have added the information whether there was any recent history of trauma (see Page 5, line 90).

Changes in the text: A 64-year-old Chinese man, who was a farmer and had previously been diagnosed with bronchiectasis in a local hospital based on chronic cough and expectoration (lasting >10 years), was admitted to our hospital after experiencing exacerbation involving nine days of rigor, moderate fever, increased cough and expectoration of white-yellow purulent sputum, as well as four days of pain in the right thigh. The patient reported no hemoptysis, chest pain, hot flashes, night sweats, obvious dyspnea, or weight loss. Prior to admission to our hospital, he had received levofloxacin (0.5 g once a day) for three days at another hospital, but this had no effect on his respiratory symptoms, fever, or thigh pain. He reported having received β -lactam antibiotics such as penicillins to treat previous exacerbations of bronchiectasis, but he was unable to provide further details about the cause of his bronchiectasis or results of microbiological tests. About three weeks prior to admission to our hospital, he had been diagnosed with gingivitis based on bleeding gums in a clinic, which had resolved after chlorhexidine gargle. He was taking amlodipine besylate (5 mg) and enalapril maleate (10 mg) once daily 5 years for essential arterial hypertension. He reported having quit smoking 10 years prior to admission to our hospital, before which he had been smoking at least 20 cigarettes per day for 30 years. He denied recent travel, surgery or trauma, and he reported no history of infectious diseases, diabetes mellitus or use of alcohol or drugs (Case presentation, paragraph 1).

Major Comment 4: It is concerning that pus culture identified *S. constellatus* but sequencing failed to identify the same organism. The authors should discuss this discrepancy in more detail and provide an explanation of the seemingly false negative sequencing result.

The authors should provide some justification for the opinion that *S. constellatus* should be considered a pathogen to exacerbation of bronchiectasis.

The references should include further publications of *S. constellatus* infections, ideally focusing on cases where infection arose in the respiratory tract, particularly the lungs.

Reply 4: Thank you for your comment. Firstly, we have quickly discussed the likely explanations for this result discrepancy between pus culture and metagenomic next-generation sequencing (see Page 8-9, line 177-180). Secondly, we have provided some justification for the opinion that *S. constellatus* should be considered a potential pathogen to exacerbation of bronchiectasis, and added some related references in this section (see Page 9-10, line 198-206).

Changes in the text: However, it failed to definitively identify *S. constellatus* in our patient, although it did detect other oral pathogens in abscess fluid. The most likely explanations include the accuracy and completeness of the sequencing database in laboratory, and the false negative caused by human omission of the detected sequences that were obviously less than those of other aforementioned oral anaerobes (Discussion, paragraph 3).

In addition, the origin of the *S. constellatus* in the thigh abscess in our patient is unclear. It seems that poor oral hygiene was the most likely cause of bacteremia due to *S. constellatus*. However, the other possibility is the lung, even if sputum culture tests were negative, because primary lung infection can lead to pyopneumothorax and pulmonary abscess (7,19,20). Indeed, this can further evidence by another study which showed the *S. anginosus* group can induce chronic pulmonary infections and was the numerically dominant pathogen at the onset of 39% of acute pulmonary exacerbations in cystic fibrosis patients (21). Those investigators recommended expanding *S. anginosus* group surveillance to bronchiectasis patients (Discussion, paragraph 5).

Minor Comment 1: A blood pressure of 92/61 in a patient presenting with sepsis is of concern and should not be described as ‘near-normal’, but is low – line 81

Reply 1: Thank you for your valuable suggestion. The “near-normal” has been changed to “low” in the revised manuscript (see Page 5, line 93).

Changes in the text: At admission, the patient had a high body temperature (38.5 °C), an increased respiratory rate (23 breaths/min), a low blood pressure (92/61 mmHg), a normal heart rate (89 beats/min), and a normal oxygen saturation in ambient air (96%) (Case presentation, paragraph 2).

Minor Comment 2: Re-phrase the term ‘weakened breath sounds’, since this is not standard terminology – line 84.

Reply 2: Thank you for your valuable suggestion. The term “weakened breath sounds” has been changed to “diminished respiration” (see Page 5, line 95).

Changes in the text: Physical examination showed **diminished respiration** and moist rales in the left lower lung (Case presentation, paragraph 2).

Minor Comment 3: The word ‘are’ should read ‘were’ – line 94.

Reply 3: Thank you for your valuable suggestion. The term “are” has been changed to “were” in the revised manuscript (see Page 5, line 107).

Changes in the text: Body temperature was monitored daily and white blood cell counts were measured every three days throughout hospitalization, until they **were** normal (Fig. 1A-B) (Case presentation, paragraph 3).

Minor Comment 4: The description of the CT appearance does not make sense and should be reworded (proximal lumen of the lode, tube walls and shadow in the local lumen)– lines 101 and 103.

Reply 4: Thank you for your valuable suggestion. We have changed the description of the CT appearance in the revised manuscript (see Page 6, line 113-114).

Changes in the text: **Computed tomography of the chest showed varying degrees of expansion and plugging in the bronchus of the left lower lobe, with slight infiltration** (Fig. 2A) (Case presentation, paragraph 4).

Minor Comment 5: Clarify the respiratory symptoms referred to in line 124.

Reply 5: Thank you for your valuable suggestion. We have clarified the respiratory symptoms in the revised manuscript (see Page 7, line 141).

Changes in the text: By four days after this change in antibiotic therapy **and eight days after drainage** began, the patient’s **cough, expectoration**, fever, and thigh pain showed significant improvement (Case presentation, paragraph 7).

Minor Comment 6: The authors should comment on whether they considered drainage of the abscess to be the primary treatment and the most influential treatment on recovery, or whether it was the use of antibiotics.

Reply 6: Thank you for your valuable comment. We think the timely and adequate drainage of pus is the cornerstone of treatment, and have added some comments in the revised manuscript (see Page 11, line 229-230; line 236-238).

Changes in the text: **Timely and adequate drainage of pus is the cornerstone of treatment.** Direct surgical incision or percutaneous catheters can be chosen for the drainage of pus, but the latter is more widely used due to its safety and high success rate (> 80%). Drainage can be performed under the guidance of ultrasonography or computed tomography (11). ~~The bacteria in the pus cultures from our patient were~~

resistant to the initial antibiotic treatment, but we were able to adjust the treatment to target the infection using a combination of ceftriaxone and vancomycin. Our patient's symptoms improved after adequate antibiotic therapy, yet we attribute the remission more to active percutaneous catheter drainage guided by color ultrasonography (Discussion, paragraph 7).

Reviewer B:

Feng Y et al. reported the first case of pyogenic thigh abscess caused by *Streptococcus constellatus* subsp. *constellatus* in a patient with exacerbation of bronchiectasis. This case report is well written, but the manuscript contains several concerns.

Major Comment 1: The timing of administration of antimicrobial agents and the timing of collection of various cultures such as blood, sputum and pus are extremely important in infectious disease practice. Please describe the date on which these cultures were collected.

Reply 1: Thank you for your valuable comment. We have described the date on which these cultures were collected in the revised manuscript (see Page 6, line 127-132).

Changes in the text: Metagenomic next-generation sequencing of the pus sample on day 3 after admission detected the presence of oral anaerobic bacteria, including oral *Prevotella* and endodontic *Porphyromanus*. Culture tests of abscess fluid on days 3, 4 and 7 after admission indicated the growth of *S. constellatus* subsp. *constellatus*, whereas *S. constellatus* was not detected in culture tests of the patient's blood on days 1, 3 and 7, and in sputum samples on days 1, 3, 7 and 9 after admission (Case presentation, paragraph 6)

Major Comment 2: The authors described, "There are no previous reports of purulent thigh infections caused by *S. constellatus*." Pyomyositis is defined as a pyogenic infection of skeletal muscle to abscess formation. Thigh pyomyositis due to *Eubacterium lentum* and *S. constellatus* from a periodontal source was previously reported (PMID: 8824994). The authors need to correct this sentence.

Reply 2: Thank you for your valuable comment. We have corrected this sentence and added this case report as a reference in the revised manuscript (see Page 3, line 63-65).

Changes in the text: We are aware of one report of purulent thigh infection caused by *Eubacterium lentum* mixed with *S. constellatus* in a patient with periodontal disease, but the *S. constellatus* subspecies was not identified (10) (Introduction, paragraph 2).

Major Comment 3: Did the gram stain of pus sample reveal monobicrobial or polymicrobial pattern? We think that this case is unclear whether monomicrobial infection or polymicrobial infection including *S. constellatus*. In general, *S. constellatus* is more likely to be polymicrobial (PMID: 11317256). Moreover, it is difficult to isolate anaerobic bacteria in pure culture for identification (PMID: 29458156). It is important to identify causative pathogen in clinical practice. Thus, the authors should provide more detail about identify method (For example, MALDI-TOF MS, Vitek 2 ANC card and so on).

Reply 3: Thank you for your valuable comment. Our pus sample revealed monobicrobial of *S. constellatus* subsp. *constellatus*. In addition, we have provided more detail about bacteria identify method in the revised manuscript (see Page 6-7, line 132-134).

Changes in the text: The pathogen was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Case presentation, paragraph 6).

Major Comment 4: P8 Line 160; The authors described, “However, the technology failed to definitively identify *S. constellatus* in our patient, although it did detect other oral pathogens in abscess fluid.” The authors need to discuss the results of this difference.

Reply 4: Thank you for your comment. We have quickly discussed the likely explanations for this result discrepancy between pus culture and metagenomic next-generation sequencing (see Page 8-9, line 177-180).

Changes in the text: However, it failed to definitively identify *S. constellatus* in our patient, although it did detect other oral pathogens in abscess fluid. The most likely explanations include the accuracy and completeness of the sequencing database in laboratory, and the false negative caused by human omission of the detected sequences that were obviously less than those of other aforementioned oral anaerobes (Discussion, paragraph 3).

Minor Comment 1: Please provide dosage and dose interval for antibiotics.

Reply 1: Thank you for your suggestion. We have provided dosage and dose interval for antibiotics in the revised manuscript (see Page 6, line 122; Page 7, line 138).

Changes in the text: Based on the underlying bronchiectasis and the possibility of suppurative infection of the right thigh, the patient was initially treated empirically with piperacillin-tazobactam (4.5 g every 8 h) and tinidazole (0.8 g once a day) therapy from the first day after admission (Case presentation, paragraph 5).

Thus, the combination of piperacillin-tazobactam and tinidazole therapy was replaced with ceftriaxone (2 g once a day) and vancomycin (0.5 g every 8 h) (Fig. 4A) (Case

presentation, paragraph 6).

Minor Comment 2: P4 Line 84; Did he have poor oral hygiene and dental caries?

Reply 2: Thank you for your comment. This patient had poor oral hygiene and dental caries, and we have provided more detail about his oral hygiene and examination in the revised manuscript (see Page 5, line 97-98).

Changes in the text: Oral examination showed several dental caries and calculus, but no obvious signs of gingivitis (Case presentation, paragraph 2).

Minor Comment 3: P4 Line 85; Did the right thigh show redness?

Reply 3: Thank you for your comment. This patient did not show obvious redness in his right thigh, and we have added this detail in the revised manuscript (see Page 5, line 97).

Changes in the text: The right thigh showed swelling, increased tension, elevated skin temperature, tenderness, limited movement but no redness (Case presentation, paragraph 2).

Minor Comment 4: P4 Line 87; Please add creatine kinase (CK) levels.

Reply 4: Thank you for your comment. We have added the creatine kinase (CK) level in the revised manuscript (see Page 5, line 104-105).

Changes in the text: Laboratory results showed a high white blood cell count (22.69×10^9 /L; normal range, $3.5-9.5 \times 10^9$ /L), a high neutrophil percentage (95%), a normal hemoglobin count (146 g/L), a high level of N-terminal pro-brain natriuretic peptide (551 ng/L; normal range, 0-227 ng/L), a high D-dimer level (1.22 mg/L; normal range, < 0.55 mg/L), a significantly high procalcitonin level (2.16 ng/L; normal range, < 0.046 ng/L), a low blood albumin level (22.9 g/L; normal range, 40-55 g/L), and a normal creatine kinase level (91 IU/L; normal range, 19-226 IU/L) (Case presentation, paragraph 3).

Minor Comment 5: P7 Line 136; Please add the reference.

Reply 5: Thank you for your comment. We have corrected and rephrased this sentence in the revised manuscript (see Page 7, line 153-154).

Changes in the text: In clinical practice, as deep tissue, thigh muscles are not uncommon sites of abscess formation (Discussion, paragraph 1).

Minor Comment 6: P7 Line 143; The authors described, “reports of *S. constellatus*-associated abscesses in other parts of the body are rare.” But, *Streptococcus anginosus* group (*S. intermedius*, *S. constellatus* and *S. anginosus*) are capable of causing serious pyogenic infections, with a tendency for abscess formation. The authors need to

correct or delete this sentence.

Reply 6: Thank you for your comment. We have deleted this sentence in the revised manuscript (see Page 8, line 160-161).

Changes in the text: As far as we know, ~~the present case is the first report of thigh abscesses caused by *S. constellatus* subsp. *constellatus* in a patient with exacerbation of bronchiectasis.~~ and reports of *S. constellatus* associated abscesses in other parts of the body are rare (Discussion, paragraph 1).

Minor Comment 7: P7 Line 147; The authors described, “It is easily missed by routine microbial analyses conducted in aerobic environments.” However, this routine analysis is performed in sputum and urine cultures in general, and aerobic and anaerobic cultures are routinely performed in blood and pus cultures. The authors need to correct this sentence.

Reply 7: Thank you for your comment. We have corrected this sentence in the revised manuscript (see Page 8, line 164-165).

Changes in the text: There is no clear understanding of the clinical burden of *S. constellatus*, due to a lack of epidemiological data. *S. constellatus* requires 5% CO₂ or an anaerobic environment to grow; therefore, it is easily missed by ~~routine~~ **microbial culture tests** conducted in aerobic environments (13) (Discussion, paragraph 2).

Minor Comment 8: P9 Line 192; This sentence “the repeated use of penicillin antibiotics for the treatment of bronchiectasis exacerbations may have caused insensitivity.” should be described in case presentation section

Reply 8: Thank you for your comment. We have described this in the case presentation section and added some related details in the revised manuscript (see Page 4, line 81-84).

Changes in the text: ~~He reported having received β-lactam antibiotics such as penicillins to treat previous exacerbations of bronchiectasis, but he was unable to provide further details about the cause of his bronchiectasis or results of microbiological tests~~ (Case presentation, paragraph 3).

Minor Comment 9: P10 Line 200; We think antimicrobial agents and drainage make his symptom improvement. Please delete this sentence “The bacteria in the using a combination of ceftriaxone and vancomycin.”

Reply 9: Thank you for your comment. We have deleted this sentence in the revised manuscript (see Page 11, line 233-236).

Changes in the text: ~~The bacteria in the pus cultures from our patient were resistant to the initial antibiotic treatment, but we were able to adjust the treatment to target the infection using a combination of ceftriaxone and vancomycin. Our patient’s~~

symptoms improved after adequate antibiotic therapy, yet we attribute the remission more to active percutaneous catheter drainage guided by color ultrasonography (Discussion, paragraph 7).

Minor Comment 10: Predisposing factors associated with pyomyositis include immunodeficiency, trauma, concurrent infection, and malnutrition, and thus the authors need to describe predisposing factors.

Reply 10: Thank you for your comment. We have described some predisposing factors in the revised manuscript (see Page 9, line 191-198).

Changes in the text: Although infections caused by *S. constellatus* can be observed in individuals with normal immunity, a growing list of conditions were identified as potential predisposing factors, such as alcohol abuse, trauma, surgery, immunosuppression, multimorbidity, rheumatic diseases, cardiovascular diseases, chronic obstructive pulmonary disease, concurrent infection, and diabetes mellitus (3,18). Our patient had chronic underlying bronchiectasis and was suffering an acute exacerbation, so further work should examine whether this condition also is a predisposing factor of *S. constellatus* infection (Discussion, paragraph 7).