To the editor, BMC Infectious Diseases.

First, thank you for revising our manuscript entitled “Pseudonocardia carboxydivorans in cerebrospinal fluid: a case report in a patient with traumatic brain injury” for evaluation as “Brief Report”. The manuscript has been revised, following the comments and improvements suggested by the reviewers. This article has not been published previously and is not under consideration by any other journal. The final manuscript has been approved by all authors.

We think this topic will be of special interest of your readers because as far as we know this is the first documented case of isolation of the germ in human cerebral spinal fluid.

We hope that the style and content of the manuscript is in accordance with the Journal requirements, but please do not hesitate to contact us should the need arise.

The required point-by-point response to the comments, is below.

Reviewer #1 Reviewer #1: I have several questions and comments:
From the article itself it is not clearly evident what was chronological order of events:

The patient received immunoglobulins, the next lumbar puncture showed better results and the patient was better clinically as well even before receiving antibiotic. There is no mention whether antibiotics were given together with immunoglobulins.

How do you explain normal results of the puncture an positive culture for Pseudonocardia later on; were there any attempts to confirm the pathogen with PCR from CSF?

Meningitis after brain trauma is usually related to neurosurgical procedure or open fracture. This was not the case in this patient. I am interested in pathogenesis of the infection and epidemiology because the ecology of this microorganism is quite specific. At the same time there is question about evolution of the disease, since trauma occurred one month before the admission to hospital and the patient had an episode of respiratory tract infections.

Since the pathogen is really unusual some speculation should be in order.

The conclusion of the article is very bold and it would be better to say that there is a possibility of connecting this microorganism with human pathology.

“From the article itself it is not clearly evident what was the chronological order of events…”

A month before admission the patient suffered a syncope episode with a traumatic brain injury (TBI). A week after the TBI, he suffered a respiratory infection and was treated with amoxicillin-clavulanic acid for 7 days. Any other antibiotic was administered before the isolation of Pseudonocardia. A lumbar puncture was carried out before the beginning of the immunoglobulins treatment. This treatment began the same day the result of electromyography was received, showing a sensory and motor mixed polineuropathy with a severe demyelination component. With this treatment the patient improved. When the isolation of Pseudonocardia we thought the bacteria could be a commensal microorganism. But the cerebral spinal fluid (CSF) is usually sterile and the isolation of bacteria might be considered as an infection. So we decided to treat the patient trimethoprim-sulfamethoxazole (SXT) during 10 months. Patient follow-up after 15 months has shown a favorable evolution. The patient declined a new lumbar puncture in the absence of symptoms.

“How do you explain normal results of the puncture a positive culture for Pseudonocardia later on; were there any attempts to confirm the pathogen with PCR from CSF?”

The characteristics of cerebral spinal fluid (CSF) improved with the treatment, as showed in the CFS control samples, but Pseudonocardia positive culture was kept on. Despite the asymptomatic patient we decided to continue the trimethoprim-sulfamethoxazole for 10 months. As we thought a long-term treatment would be required for its eradication.

PCR from CSF was not attempted as the patient declined a new lumbar puncture in the absence of symptoms.
“Meningitis after brain trauma is usually related to neurosurgical procedure or open fracture. This is not the case of this patient. I am interested in the pathogenesis of the infection and epidemiology because of the ecology of the microorganism is quite specific…”

We couldn’t know exactly how the infection was acquired. Carboxydothobia are a group of aerobic bacteria, which have been isolated in soil samples.

The patient suffered a syncope episode while cultivating vegetables and he suffered a traumatic brain injury (TBI), but with no neurosurgical procedure or open fracture. Pseudonocardia could have developed in the soil he cultivated and get in contact with the patient in the traumatic brain injury. The respiratory tract infection might have been another gate of entrance, since Pseudonocardia is a well-known antifungal commensal and a higher abundance of this taxon might rather reflect the presence of fungal organism in the distal airways. But this patient had not had any fungal infections, nor breathing or rheumatic pathologies, so we though this way of entrance would be unprobable.

“Since the pathogen is really unusual some speculation should be in order. The conclusion of the article is very bold and it would be better to say that there is a possibility of connecting this microorganism with human pathology.

To the best of our knowledge, this is the first report of an isolation in the cerebral spinal fluid of Pseudonocardia carboxydivorans. The presence of this germ in the cerebral spinal fluid, usually sterile, in addition of neurological symptoms and the obtained laboratory results among the 4 CFS sample suggest meningitis and not the presence of a commensal microorganism. Because of its exceptionality we decided to publish it. Accurate identification of Pseudonocardia carboxydivorans would further help investigations defining its pathogenic role in human infections.

Minor comments:

sex of the patient (in the text the patient is referred as he but on page 4, line 42 it is written "her body..")

normal laboratory values are missing; CRP in my opinion was only slightly elevated

there are some spelling mistakes of microorganisms -- eg. Citomegalovirus

the results of MR examination should be reviewed

Mistakes were corrected

This writing is made to make the necessary clarifications.
1. Nuclear magnetic resonance images performed in May 2013 has been reviewed in conjunction with the Radiology Service. No findings have been found to be added to those previously described.

2. The patient received treatment with trimethoprim-sulfamethoxazole from 02/07/2013 to 07/17/2014. He did not receive any other antibiotic treatment added.

3. He received intravenous immunoglobulins (5 doses) starting the first dose on the day the electromyogram result was received.

4. With respect to the analytical for which information is requested:

   - Biochemistry: glucose 197 mg / dL (values within normal range 74-109),
   - C-reactive protein 11.8 mg / L (values within normal range 0-5)
   - Hemogram: total hemoglobin 14.4 g / dL (values within normal range between 13.5-18), erythrocyte sedimentation rate 51 mm 1h (values within normal range 1-20).

Review #2

Major Point: It is certain that the isolated bacterium from a patient is a group of genus Pseudonocardia. However, it is not clear that the isolate is a Pseudonocardia carboxydivorans. The authors only showed the similarity of 16S rRNA between the isolate and P. carboxydivorans and the similarity was a 99.7% not a 100%. Since the 16S rRNA sequence was not exactly same to P. carboxydivorans, it can be a new strain. For example, according to Park et al., (2008), even though the similarity of 16S rRNA between P. carboxydivorans and Psedonocardia alni was 99.6%, but it turned out a new strain, P. carboxydivorans. To clear this possibility, I recommend to clone and sequence the CO-DH gene by the method of Park et al., (2008) from the isolate because CO-DH gene is the unique characteristics of P. carboxydivorans to distinguish it from the other Pseudonocardia. If the CO-DH gene is cloned and it is same to that of P. carboxydivorans, I agree the isolate is a P. carboxydivorans. If not, the authors should try to identify the isolate.


The gold standard method for species differentiation of the genus Pseudonocardia is the sequencing of the 16S RNA gene. The percents of 99.7% similarity to the type sequence of the species P. carboxydivorans, is higher than that required to differentiate species of Aerobic Actinomycetes. In a few cases in our experience of using this gene in more than 15,000 isolates, in a very low proportion we identify the strains with 100% similarity. In a series of phenotypic tests, which were not included in the text, the strain's membership to P. carboxydivorans was
corroborated: Production of Acid from Inulin and the negativity of the production of acid from fructose, maltose, sorbitol and xylose. in adition to negative growth in 5% ClNa, clearly differentiate our strain of the P. alni type species.

On the other hand, in the study by Park (1989) that the CO-DH gene is not cloned, to differentiate species from Pseudonocardia, another study in which an immunoenzymatic assay is performed (Kraut et al., 1989, Arc. Microbiol 152, 335-241.), Whose methodology is beyond the scope of the case report that concerns us.

Minor points:

1. There are so many confusions periods and commas. For example, in page 5, 1,44 g/l should be changed to 1.44 g/l; 0.88 g/l protein to 0.88 g/l; 5.9 u/l to 5.8 U/l. I found similar errors on page 6 and table 1.

2. It seems that this manuscript was written without prudence. I am able to find so many errors of duplicated periods by mistake, word spacing, confusion of periods with commas and so on... For example, in title page, in KEYWORDS, there is double periods before carboxydobacteria; AUTORS should be changed to AUTHORS; there are also errors in author's affiliations in word spacing, confusion of periods with commas.

3. Is it fine to use a personal email address instead of the official email address of institution for the corresponding author?

4. This sentence "Members of the genus Pseudonocardia have been widely reported and recovered from several ecosystems, such as soil samples and plant samples. Pseudonocardia bacteria colonize the microbial communities on the integument of fungus -gardening ant species" repeated 3 times in this manuscript. Please delete the last one in discussion.

5. It will be more informative introduction if authors are able to provide the reports of human infection by genus Pseudonocardia.

6. The line numbers on left were not match to the manuscript. I'm not sure it is only my problem due to a software version.

The points 1 to 6 were revised and corrected.