
This is the **accepted version** of the letter:

Nuez-Zaragoza, Elisa; Sanfeliu Sala, Isabel. «Acute necrotizing chorioamnionitis caused by *Actinomyces neuui*». *Enfermedades infecciosas y microbiología clínica*, Vol. 40, Num. 8 (October 2021), p. 458-459 DOI 10.1016/j.eimc.2021.06.017

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Enfermedades Infecciosas y Microbiología Clínica

Acute necrotizing chorioamnionitis caused by *Actinomyces neuui*

Corioamnionitis aguda necrotizante causada por *Actinomyces neuui*

--Borrador del manuscrito--

Número del manuscrito:	EIMC-D-21-00251R1
Tipo de artículo:	Cartas al Editor
Autor correspondiente:	Elisa Nuez-Zaragoza Corporació Sanitària Parc Tauli: Consorcio Corporacion Sanitaria Parc Tauli Sabadell, Barcelona SPAIN
Primer autor:	Elisa Nuez-Zaragoza
Orden de autores:	Elisa Nuez-Zaragoza
	Isabel Sanfeliu
Respuesta a los revisores:	1-In the title, the authors state that is a "severe" chorioamnionitis, but no in the text. The patients should unify the criteria and also shortly explain why it is a "severe" chorioamnionitis. We decided to change the word "severe" by acute necrotizing chorioamnionitis, as the pathology department showed. 2-Line 16: please remove "which 5 days before.....mg/dl". This sentence has been removed, and we only comment the value of the CRP at the last blood test. 3-Line 33: What culture media were used? All the culture media used have been added to the text. 4-What is the value that the authors gave to the growth of <i>S. anginosus</i> in this culture? What could be the explanation why in the Gram stain could not be observed Gram positive cocci? About the value of <i>S. anginosus</i>, we added a paragraph explaining the reasons about our considerations of its value and why it was not seen in the Gram stain. 5-The authors should add the log score obtained for the identification of <i>A. neuui</i> and <i>S. anginosus</i> in both samples. According with this score, an additional technique could be used for the final identification. The log score from MALDI is added next to the name of the identified bacteria. In the case of <i>Actinomyces neuui</i> there is a slight difference in the log score between placenta and amniotic fluid samples. 6-Regarding to the susceptibility testing, to our best knowledge, there is no a specific panel for <i>Actinomyces</i> in MicroScan, so the authors should specify what kind of panel have them used to obtaining the MIC. The panel we used was Pos MICroSTREP plus 6 panel, MicroScan WalkAway which includes antibiotics for gram positive bacteria and also it is used for the growth of fastidious microorganisms. 7-Page 2, lines 16-21: this paragraph should be removed and placed on the clinical history. The paragraph is moved as requested. We placed it in the clinical history and we also added there the pathology result. 8-The manuscript should be reviewed by a native English speaker. The manuscript has been reviewed by a native English speaker.

Sabadell, 4th May 2021

Dear Editor,

Please find enclosed the case report entitled "Severe chorioamnionitis caused by *Actinomyces neuui*", that we submit to you to be considered for publication in *Enfermedades Infecciosas y Microbiología Clínica*.

This case describes a case of severe chorioamnionitis caused by *Actinomyces neuui*, a bacteria present in the female genital tract that can cause infections in some clinical situations as the usage of vaginal devices. This case shows the importance of studying the amniotic fluid in cases of suspected chorioamnionitis with the aim of making an early diagnosis that can avoid further infective complications to the mother and the newborn.

The Authors warrant to *Enfermedades Infecciosas y Microbiología Clínica* that the case has not been previously published, submitted, or accepted for publication elsewhere and that each author has contributed equally to this letter and has approved the final version.

Yours sincerely,

Elisa Nuez-Zaragoza (Corresponding author)

Isabel Sanfeliu

Body text word count: 705

Number of figures: 1

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Elisa Nuez-Zaragoza

Acute necrotizing chorioamnionitis caused by *Actinomyces neuui*

We present the case of a 41-year-old female patient who was admitted to the hospital at week 25 of her pregnancy for uterine dynamics and foetal lung maturity control. After five days of hospitalization, she began treatment with atosiban (oxytocin antagonist) due to increased uterine dynamics. Previous significant medical history presented in vitro fertilisation and gestational diabetes with insulin treatment. A cerclage was placed 22.3 weeks into the pregnancy due to prolonged cervical length.

During the hospitalization (week 27.5), the patient began experiencing an increased clear vaginal discharge without uterine dynamics resulting in a premature rupture of membranes. Laboratory blood test revealed an **increase in C-reactive protein of 5.47 mg/dL (0-0.5 mg/dL)**. Based on the findings, a presumptive diagnosis of chorioamnionitis was made leading to amniocentesis for study of amniotic fluid.

The results of the amniotic fluid study were as follows: glucose <2 mg/dL, proteins 4 g/L and a count of 1720 nucleated cells/mm³ with a predominance of polymorphonuclear cells. The Gram stain showed unbranched Gram-positive bacilli (Fig. 1).

Based on the laboratory data, the patient was diagnosed with chorioamnionitis starting with antibiotic dosage (intravenous piperacillin-tazobactam and oral clarithromycin). The obstetrician decided to induce labour and stopped the atosiban bolus. A baby weighing 1200 gr was born by spontaneous delivery at week 28.

The anatomical pathology study of the placenta showed acute necrotizing chorioamnionitis but the patient did not exhibit signs of fever or sepsis after delivery, and the antibiotic treatment was concluded 24 hours postpartum. Due to the premature birth, the new-born was admitted to paediatric intensive care and was treated with ampicillin and gentamicin. Antibiotic treatment concluded after 5 days without further signs of bacterial infection.

Microbiological studies used amniotic fluid and two placenta samples. All the samples were cultured in selective (**MacConkey and Columbia CNA agar**) and enrichment media (**chocolate agar, blood agar, and thioglycolate broth**) in aerobic conditions. We also used **Anaerobe Schaedler and Schaedler neomycin vancomycin agar** under anaerobic conditions (**bioMérieux®**). Two types of colonies were isolated in the amniotic fluid culture. Some colonies had a circular and smooth white colour and were identified as *Actinomyces neuui* by mass spectrometry (**log score: +2.06, MALDI-TOF MS, Microflex®, Bruker**). The other colonies were smaller and darker and were identified as *Streptococcus anginosus* by MALDI-TOF MS (**log score: +2,16**) as well. In both placenta samples, only *Actinomyces neuui* was isolated and identified (**both log score: +2.16**).

Susceptibility testing used the minimum inhibitory concentration (MIC) microdilution method (**Pos MICroSTREP plus 6 panel, MicroScan WalkAway, Beckman Coulter**). The sensitivity analysis showed that both strains were susceptible to all antibiotics tested: ampicillin *A. neuui* (<=0.06 mg/L)/*S. anginosus* (0.12 mg/L), cefotaxime (<=0.25 mg/L), meropenem (<=0.25 mg/L), levofloxacin *A. neuui* (1 mg/L)/*S. anginosus* (<=0.5 mg/L), clindamycin (<=0.06 mg/L), clarithromycin (<=0.12 mg/L), vancomycin (0.5 mg/L), and daptomycin (<=0.25 mg/L).

***Streptococcus anginosus* was not seen in the Gram stain, and it grew at low CFU counts in the inoculated amniotic fluid samples. The placenta samples did not reveal its presence; thus, it was considered to be a contaminant of the sample extraction.**

Actinomyces neuui is a facultative anaerobic Gram-positive and rod-shaped bacteria. It has an unbranched morphology in contrast to other species of *Actinomyces*, (1-3). *Actinomyces* species are considered normal flora of the oral cavity, gastrointestinal tract, and female genital tract; they rarely cause disease in humans (3-8). Nevertheless, they can cause chronic and slowly progressive infections including abscess formation in skin and soft tissues (4,6,9). *Actinomyces neuui* is a lesser-known species first described in 1994 by Funke et al. (2). It does not cause typical actinomycosis. The infections are related to abscesses, skin lesions, and infections of the genitourinary tract and mostly associated with the use of intrauterine contraceptive devices (IUD) (1,4,7-10). Rare cases of pericarditis, osteomyelitis, breast infections, ophthalmic infections, prostatitis, and bacteraemia have also been previously reported. (1,3,5-8).

Actinomyces neuui has also been involved in causing premature labour, chorioamnionitis, and neonatal sepsis associated with a previous resolution of a vaginal device (IUD or vaginal cerclage). (1,4-10)

In our patient, chorioamnionitis was suspected to be related to the cerclage placement at week 22 of pregnancy. Other authors reported similar conclusions of *Actinomyces neuui* infections associated with the use of this device (1,8). **Only two cases reported the presence of *Actinomyces neuui* in amniotic fluid (5,10).**

This case demonstrates the need to consider *Actinomyces* infection in patients who carried a vaginal device. Amniotic fluid should be examined in patients with suspected chorioamnionitis for an early diagnosis to avoid further complications to the mother and the new-born.

Key words: *Actinomyces*, chorioamnionitis, amniotic fluid, cerclage

Conflict of interest

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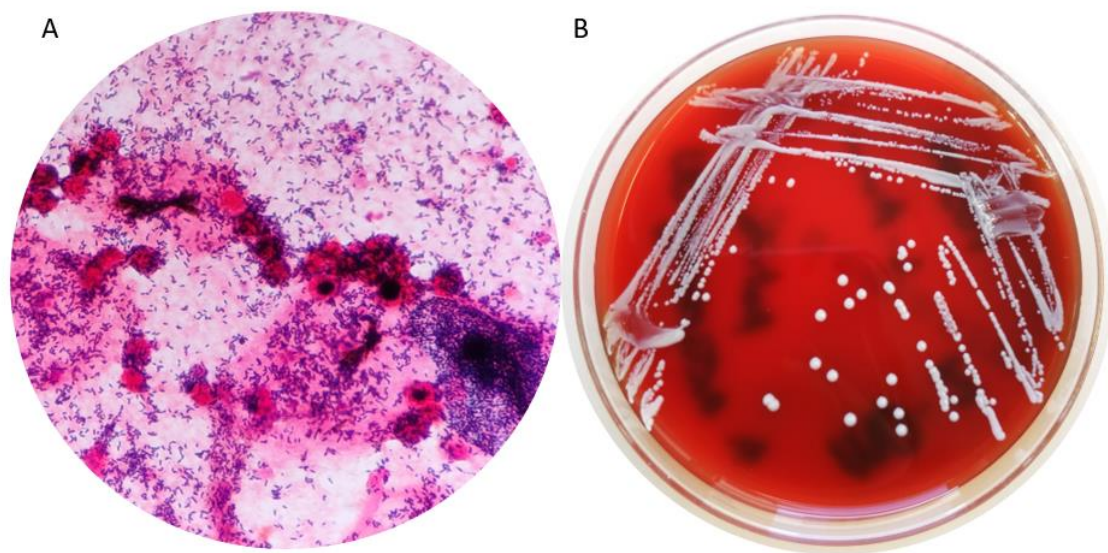
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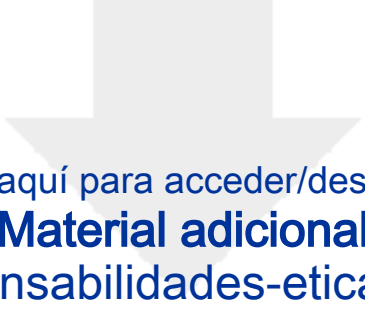
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Fig.1. Direct Gram stain from the amniotic fluid revealed unbranched gram-positive bacilli (A). White colonies of *Actinomyces Neuui* isolated on Blood agar after 48 h of incubation (B).





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