There are immunochromatography methods that enable the detection of carbapenemases from isolated colonies where the sensitivity and specificity are 100%[4]. There are no reports on the use of such methods on direct samples in our setting, and the costs of these determinations are similar to those of molecular methods.

There is evidence of the use of BD MAX™ on direct blood culture samples for the detection of methicillin-resistant *Staphylococcus aureus*[5–7]. In these studies, sensitivity and specificity were in the range of 98%–100%.

To our knowledge, there are no reports in the literature evaluating the BD MAX™ Check-Points CPO assay using direct samples. Our study has shown that the assay detected 100% of the *bla*KPC, *bla*NDM and *bla*OXA-48 carbapenemase-producing Gram-negative bacilli compared to traditional culture methods. One limitation of our study is the fact that because of the low incidence in our environment no *bla*VIM/*bla*IMP was detected in the period analysed.

In this study we analysed the efficiency of the BD MAX™ Check-Points CPO method in the detection of *bla*KPC, *bla*NDM and *bla*OXA-48 in direct samples from blood culture bottles in an estimated time of 3 h.

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Disseminated *Mycobacterium avium* complex infection: microbiological confirmation by «percutaneous» sputum induction following the intracavitary instillation of normal saline☆

Infección diseminada por Mycobacterium avium *complex: confirmación microbiológica mediante inducción «percutánea» de esputo tras instilación intracavitaria de suero salino*

Dear Editor,

The incidence and morbidity and mortality rates of diseases caused by non-tuberculous mycobacteria (NTM) have been on the increase over the last twenty or thirty years.[1] There are a number of predisposing factors for the development of NTM infections, including chronic obstructive pulmonary disease, having had tuberculosis, bronchiectasis, immunodeficiency, cancer and diabetes, although no risk factors are identified in a significant proportion of patients.[2,3] Although the lung is the most commonly affected organ, NTM can also infect bones, lymph nodes, joints and the skin.[4] Most NTM lung infections are diagnosed by sputum examination and culture.[5]. If NTM cannot be isolated from expectorated secretions, the next step is usually to perform bronchoalveolar lavage (BAL) or even a lung biopsy.[1,5] We present the case of an immunocompetent patient with a multifocal infection (vertebral and lung) caused by *Mycobacterium avium* complex (MAC) in which the first microbiological confirmation was achieved after percutaneous instillation (with radiological control) of a cavitary lung lesion and provocation of induced sputum.

This was a 75-year-old man with a history of high blood pressure, dyslipidaemia and atrial fibrillation (but without any of the predisposing factors outlined in the previous paragraph), who consulted with a five-month history of asthenia, dry cough and progressive low-back pain, whose magnetic resonance imaging (MRI) and computed tomography (CT) scan of the lumbar spine showed signs of spondylodiscitis of the intervertebral spaces L4-L5 and L5-S1 (Figs. 1A and B). The chest X-ray showed opacities of infectious appearance in the right lung, with some cavitary lesions in the right lower lobe (RLL). The chest CT scan confirmed centrilobular nodules and signs of infectious bronchiolitis (Fig. 1C), as well as several cavitary lesions in the RLL, suggesting an active infection by *Mycobacterium tuberculosis* (*M. tuberculosis*) (Fig. 1D). BAL and several attempts at induced sputum (after inhalation of hypertonic saline solutions) did not demonstrate the presence of acid-fast bacteria (AFB) by auramine staining or of *M. tuberculosis* genetic material by nucleic acid amplification (Xpert® MTB/RIF Ultra, Cepheid). A percutaneous biopsy with radiological control of the L5-S1 intervertebral disc was performed (Fig. 1E), also with negative results. In the absence of microbiological confirmation, and pending the results of the specific cultures for mycobacteria (VersaTREK®, Thermo Fisher Scientific), it was decided to perform a percutaneous puncture-aspiration (with radiological control by CT) of one of the cavitary lesions in the RLL (Fig. 1F) after the instillation of about 10 ml of saline in one of the cavitary lesions. However, only 1 ml of blood-stained fluid could be aspirated. At the end of the procedure, the patient expectorated abundant thick sputum (15 ml) onto a sterile cloth (used to place the material used during the instillation procedure). There were no complications in the follow-up images taken immediately after the procedure.

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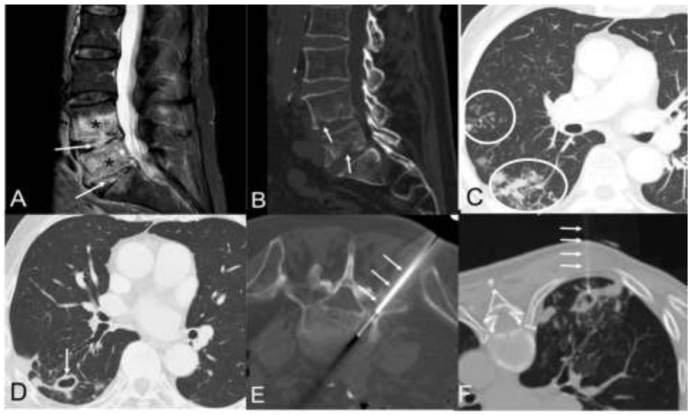

Figure 1. A) Sagittal MR image of the lumbar spine (STIR sequence) showing a marked alteration in the signal intensity of the intervertebral discs L4-L5 and L5-S1 (arrows) and the adjacent vertebral endplates (asterisks). B) Sagittal reconstruction of lumbar spine CT (bone window) which shows the bone erosions of the vertebral endplates (arrows). C) and D) Axial CT images of the chest (lung window) showing centrilobular lung nodules and "tree-in-bud" images (C, circles) and a dominant thin-walled cavitary lesion (D, arrow). E) Axial CT image of the lumbar spine (bone window, patient placed in the prone position) showing the percutaneous biopsy (arrows) of disc infection foci. F) Axial CT image of the chest (lung window, patient placed in the prone position) showing saline instillation into a cavitary lung lesion (arrows). After the instillation of 10 ml of saline, only 1 ml of fluid could be aspirated, although the patient expectorated abundant sputum (15 ml) at the end of the procedure.

The expectorated sputum was aspirated with a sterile syringe and placed in a test tube for immediate microbiological processing, confirming the presence of 1,500 AFB/100 fields in the auramine stain. Both the staining of the small amount of fluid aspirated from the cavitary lesion and the test to detect *M. tuberculosis* genetic material in the two samples were negative. The growth of MAC in the sputum obtained after the above procedure after 16 days of incubation confirmed the aetiological agent. The microbiological diagnosis was confirmed after the subsequent late growth of MAC in one of the previously obtained respiratory samples (the BAL); the cultures of the vertebral biopsy and of the rest of the respiratory samples were negative. The patient was treated with clarithromycin, ethambutol and rifampin, and both the spondylodiscitis and his lung infection responded well to the treatment.

Demonstrating NTM lung infections microbiologically is not easy, and even when they are detected, patients will not necessarily be started on treatment.[6] Among the most common diagnostic techniques are the study of sputum (spontaneous or induced), BAL, lung biopsy (transbronchial or surgical) and percutaneous aspiration of a cavitary lung lesion.[6–8] Percutaneous aspiration with radiological control of cavitary lung lesions is a cost-effective diagnostic technique for the detection and culture of multiple microbiological agents and makes it possible to differentiate between fungal infections, bacterial abscesses and mycobacterial infections.[9–11] In our case, 10 ml of saline solution was instilled into a cavitary lung lesion, but only 1 ml of the instilled fluid was aspirated, as the fluid we introduced was drained by the bronchi communicating with the lung cavity. Fortunately, the patient expectorated the percutaneously instilled saline at the end of the procedure, enabling direct detection of AFB by staining and subsequent confirmation of MAC infection by culture. Several days later, MAC growth was confirmed in one of the respiratory samples obtained initially. We were unable to find any instance in the scientific literature of microbiological demonstration of an NTM infection in ïnducedšputum occurring after percutaneous instillation (with radiological control) of saline in a cavitary lung lesion. There is one published report in which saline was instilled in a cavitary lung lesion and then aspirated back through the same needle followed by BAL, with the causal microorganism being confirmed in both samples (the fluid aspirated directly from the cavitary lesion and from the BAL).[12] We believe that this ṗercutaneousšputum induction technique may be useful in certain patients with cavitary lung lesions in whom microbiological confirmation has not been achieved using other more conventional diagnostic methods (induced sputum, BAL).

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Acute necrotizing chorioamnionitis caused by *Actinomyces neuii*

Corioamnionitis aguda necrotizante causada por Actinomyces neuii

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/mm^3 with a predominance of polimorfonuclear 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 g 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 h 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 thioglicolate 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 neuii* 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 neuii* was isolated and identified (both log score: +2.16) (Fig. 1).

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. neuii* (≤0.06 mg/L)/*S. anginosus* (0.12 mg/L), cefotaxime (≤0.25 mg/L), meropenem (≤0.25 mg/L), levofloxacin *A. neuii* (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 neuii 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 infec-

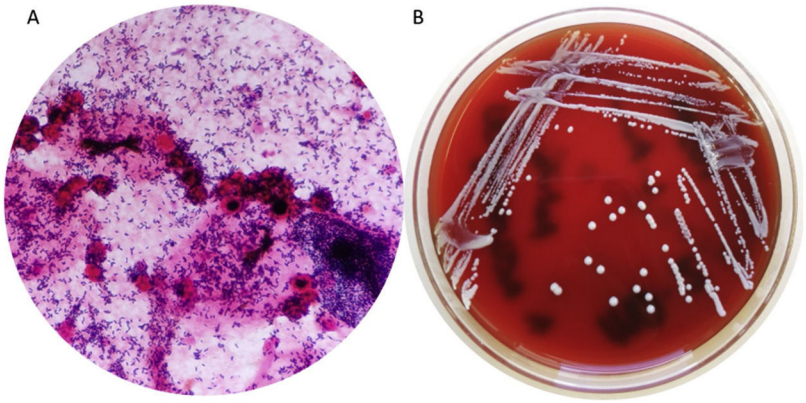

Fig. 1. Direct Gram stain from the amniotic fluid revealed unbranched gram-positive bacilli (A). White colonies of *Actinomyces neuii* isolated on blood agar after 48 h of incubation (B).