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Scientific letter

Rapid detection of *bla*KPC, *bla*NDM and *bla*OXA-48 genes in positive blood culture broths☆

Detección directa de genes bla*KPC*, bla*NDM y* bla*OXA-48 a partir de hemocultivos positivos*

Bacteraemia is associated with high morbidity and mortality rates, so it is essential to have an accurate diagnosis and an early introduction of effective antibiotic therapy[1].

As the production of carbapenemases is one of the main mechanisms of resistance in Gram-negative bacilli, methods are needed for rapid carbapenemase detection directly from clinical samples.

The BD MAX™ Check-Points CPO assay is an automated qualitative real-time PCR designed to detect the carbapenemase genes *bla*KPC, *bla*NDM, *bla*VIM/*bla*IMP and *bla*OXA-48 from swabs from patients with suspected rectal colonisation.

The objective of this study was to assess the performance of the BD MAX™ Check-Points CPO assay directly on blood culture bottles positive for Gram-negative bacilli. We studied patients admitted with suspected bacteraemia and with risk factors for infection by carbapenemase-producing Gram-negative bacilli. The risk factors considered to be associated were: history of admission in the previous 30 days; patients referred from other healthcare institutions; history of colonisation or previous infection with carbapenem-resistant Gram-negative bacilli.

For positive culture bottles where the Gram stain showed the presence of Gram-negative bacilli, we performed the conventional culture and the BD MAX™ Check-Points CPO assay simultaneously. The conventional culture plates were incubated for 18 h at 37 °C. The identification and antibiotic sensitivity of the isolates were studied using the Phoenix™ automated system (Becton Dickinson). The tests and interpretation of the results were carried out according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI)[2]. Phenotypic screening for carbapenemases was carried out using synergy with boronic acid and EDTA discs (Britania), and the CARB BLUE Kit® (ROSCO) method[3].

To perform the BD MAX™ Check-Points CPO assay, 50 µl was taken from the bottle, a 1/50 dilution was made, 10 µl of which was then entered into the kit's sample buffer. The test was then performed following the manufacturer's instructions.

A total of 59 flasks positive for gram-negative bacilli were studied, from which 61 isolates were recovered by conventional culture.

A total of 33 isolates were non-susceptible to carbapenems by culture. In 21, the presence of carbapenemases was detected by inhibition with boronic acid (19), EDTA (2) and/or the CARB BLUE Kit® method.

The following results were obtained with the BD MAX™ Check-Points CPO: 32 negatives and 29 positives (17 *bla*KPC, 8 *bla*OXA-48, 2 *bla*NDM and 2 were positive for both *bla*KPC and *bla*OXA-48) (Table 1). Only one strain was positive for OXA-48, with no evidence of resistance to carbapenems.

The results showed that BD MAX™ Check-Points CPO detected 100% of the carbapenemase-producing strains, *bla*KPC, *bla*NDM and *bla*OXA-48. The only case where the results of the culture and BD MAX™ Check-Points CPO differed was a strain of *Providencia stuartii*, in which *bla*OXA-48 was detected with no evidence of resistance to carbapenems, although this could be due to the lack of expression of the enzyme in said isolate.

Table 1
Results obtained with the direct BD MAX™ Check-Points CPO assay of positive blood cultures.

Germ	No.	Culture		BD MAX™			
		No sensitivity to carbapenems	Sensitivity to carbapenems	*bla*KPC	*bla*OXA-48	*bla*NDM	*bla*KPC *bla*OXA-48
Klebsiella pneumoniae	31	25	6	17	4	2	2
Escherichia coli	9	–	9	–	–	–	–
Acinetobacter baumannii	4	4	–	–	–	–	–
Enterobacter cloacae	3	2	1	–	2	–	–
Proteus mirabilis	3	–	3	–	–	–	–
Pseudomonas aeruginosa	3	–	3	–	–	–	–
Klebsiella oxytoca	2	–	2	–	–	–	–
Providencia stuartii	2	–	2	–	1	–	–
Serratia marcescens	2	1	1	–	1	–	–
Aeromonas veronii	1	1	–	–	–	–	–
Citrobacter koseri	1	–	1	–	–	–	–
Total	61	33	28	17	8	2	2

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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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