Modified procedure for Carba NP detection of carbapenemase-producing bacteria directly from blood cultures

A. Mazzariol[1-2], C. Castagna[1], A. R. Centonze[2], M. Parisato[2], G. Petrosino[1], M. Bragantini [2], L. Maccacaro[2] and G. Cornaglia[1-2]

[1]Dipartimento di Patologia e Diagnostica, Università degli Studi di Verona [2]Dipartimento di Patologia e Diagnostica, Azienda Ospedaliera Universitaria Integrata di Verona

Introduction:

The Carba NP test (1) is a novel phenotypic method developed for carbapenemase detection. It is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange). This test performed very well to detect carbapenemase-producing strains directly from isolated colonies growth in different media.
To notify as soon as possible clinicians of the presence of carbapenemase-producing strain in a positive blood culture, Dortet et al (CMI 2013) (2) proposed an extraction protocol from spiked (simulated) blood culture, thus applying the carba NP to detect these strains.
In this study we check the detection of carbapenemase-producing strains by applying the extraction protocol and the Carba NP test not to simulated blood cultures but directly to real clinical specimens.

Materials and Methods

Our study included twenty-five blood cultures positive for carbapenemase-producing strains obtained at the Microbiology Laboratory of Verona between March and September 2013. Twenty-two isolates were KPC producers (21 *K. pneumoniae* and 1 *E. coli*) and 3 were *P. aeruginosa* VIM producers.
All isolates proved positive with the Carba NP test performed directly from the colonies (1)
All enzymes were characterized by PCR with a set of screening primers and conditions (3) .
The Carba NP test was also directly applied to all these samples by following the extraction protocol proposed by Nordmann *et al.* (3) for 'spiked' (simulated) blood cultures. Briefly the protocol consisted in a 3-h incubation (37°C) of 20 drops of positive blood culture in 20 ml BHI with 0.125µg/ml and $ZnSO_4$ 70µg/ml. The culture obtained was centrifuged 15 min at 2500 rpm and the pellet suspended in 150 µl of 20 mM lysis buffer (B-PERII, Thermo Scientific, Pierce) and vortex with microbead (Ultraclean microbial DNA isolation Kit Bead Tubes, MO BIO laboratories) for 30 min.
Revised extraction protocol consisted of plating three drops of positive blood culture on MHA medium. Plates were incubated at 37°C and every hour (starting two hours after the inoculation) the bacteria were collected with a swab, suspended in 1 ml of saline solution, centrifuged at 8000 rpm for 5 min. The pellet was suspended in 100 µl of lysis buffer (B-PERII, Thermo Scientific, Pierce) and tested with Carba NP as for isolated colonies.
The two protocols were also applied to "spiked" cultures simulated with the same strains of clinical blood cultures.

Results

Only three specimens out of 25 resulted positive when the Carba NP test was performed on the original blood cultures following the extraction protocol proposed by Dortet (3).
To verify that this protocol was working as proposed by the authors we follow their indications for "spiked" blood culture using the same isolates that we obtained from original blood culture. In this case all 25 samples resulted positives using the extraction protocol proposed by Dortet (3).
To understand the difference between "spiked" and original blood culture we performed an unity forming colony (UFC) counting for both type of sample. The results are reported in Figure 1.
The only three positive results obtained with the original protocol contained 10^8 UFC, whilst the UFC counts of the Carba-NP-negative blood cultures ranged between 10^4 and 10^8/ml and the UFC counts of spiked cultures ranged between 10^9 and 10^{12}/ml (Figure 1).
We repeated the experiments using a modify extraction protocol, as reported in method section, for both "spiked" and original blood cultures for all the 25 samples under study. For all samples were registered the time occurred to obtain a positive carba NP test.
Using our modified protocol, 76% of the original blood cultures proved positives after 4 hours of incubation and 100% after 6 hours. (Figure 2)
Using our modified protocol, 68% of the "spiked" blood cultures proved positives after 2 hours of incubation and 100% after 3 hours (Figure 3) confirming that positivity is bacteria concentration dependent.

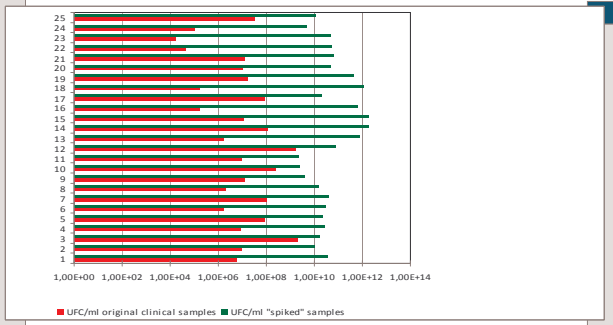

Figure 1: UFC counting for the spiked and simulated blood cultures of 25 samples under study.

Conclusions

- By using a modified extraction protocol, we could successfully apply the Carba NP test directly to 25 blood cultures of clinical origins that had proved positive for carbapenemase-producing strains.
- The need for modifying the protocol successfully applied by Nordmann *et al.* to "spiked" blood cultures is possibly related to the fact that real specimens can hardly reach a number of UFC/ml sufficient to perform the original test.
- The modified extraction protocol is very easy to perform in every microbiology laboratory.

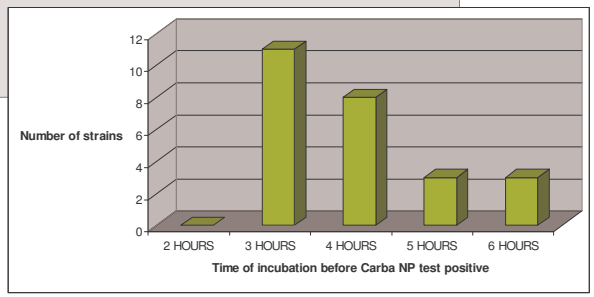

Figure 2: number of the strains that were positive at carba NP test at each time using a modified protocol for original blood cultures.

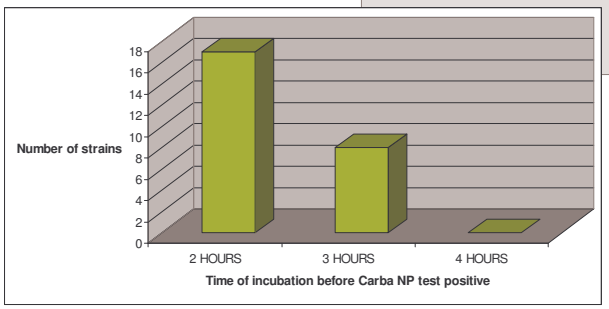

Figure 3: number of the strains that were positive at carba NP test at each time using a modified extraction protocol for "spiked" blood culture.

References

1. Nordmann EID 2012
2. Dortet CMI 2013
3. Dallenne JAC 2010