Rapid carbapenemase- and ESBL-producing strains detection from positive blood culture by using CarbaNP and ESBL-NDP tests

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Aim of the sudy

Recently, a biochemical test (Carba NP test) based on the colorimetric detection of carbapenemase production has been developed to identify carbapenemase producers from isolated colonies and from spiked blood culture (1-2).
This study aims at optimizing the application of CarbaNP test and ESβL NDP test directly from positive blood culture samples, with a new flowchart that reduces to at few hours the turnaround time for identification of carbapenemase- and ESBL-producing strains.

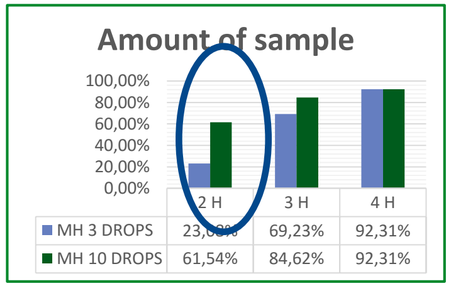
Material and Methods

A total of 35 positive blood cultures were included in the study, namely 22 for *Klebsiella pneumomoniae* and *Escherichia coli* ESBL-producing strains (SHV-12 and CTX-M15), and 13 for *K. pneumoniae* carbapenemase–producing strains (KPC).
All isolates underwent identification by Maldi-TOF, antimicrobial susceptibility test by microdiluition, PCR for detection of ESBL and carbapenemases genes (3), carbaNP and ESBL-NDP tests. (1-2)
CarbaNP and ESBL-NDP were applied to positive blood cultures under different sub-cultures conditions, namely: amount of blood to be subcultured (3 drops vs 10 drops); type of culture media (Muller-Hinton agar vs blood agar); incubation conditions (air vs 5% of CO_2). All subsets were evaluated for the CarbaNP and ESBL-NDP positivity at 2, 3 and 4 hours of incubation.

Results

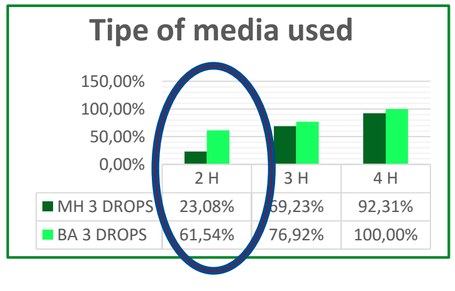
10 blood drops of positive blood cultures harbouring carbapenemases producing strains streaked on MH plates and incubated at 37° C on air yielded a 61% positivity of carbaNP test after 2 h, as compared to 23% with 3 drops; in both cases positivity raised up to 92% when the test was performed after 4 hours (graph 1)

Graph 1



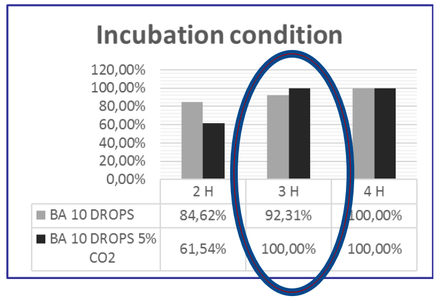
3 blood drops incubated at 37° C on air yielded after 2 hours a 61% and 23% positive CarbaNP test for blood agar and MH, respectively. At 4 hours positivity was 100% for blood agar and 92% for MH (graph 2)

Graph 2



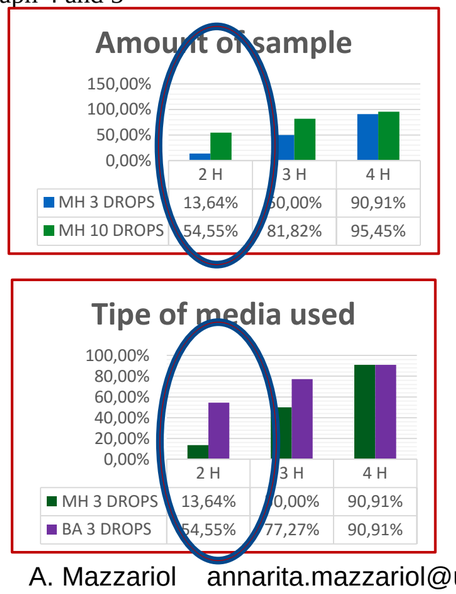
The evaluation of air incubation vs air with 5% of CO_2, performed on blood agar with 10 drops (i.e., the best conditions stemming from the previous results) showed a 100% of positive carbaNP test at 3 hours for air plus 5% of CO_2, compared to 92% only on air, and a 100% positivity at 4 hours for both. (Graph 3)

Graph 3

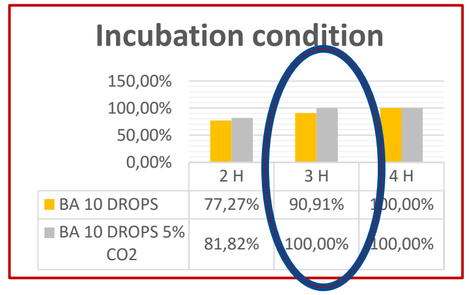


Results for all beta-lactamase producing strains (tested with the ESBL NDP test) paralleled those described above for the strains resistant to carbapenems. (Graphs 4-5-6)

Graph 4 and 5

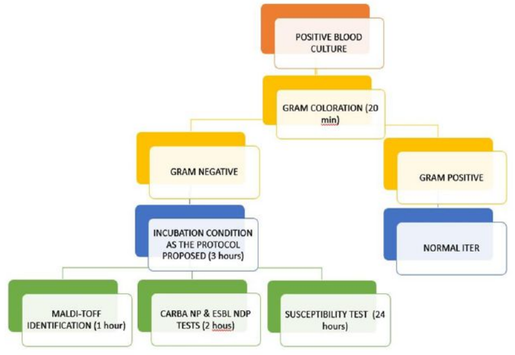


Graph 6



Conclusions

The Carba NP and ESBL NDP test were optimized for direct performance from positive blood cultures.
The best results were obtained with 10 drops of positive blood culture streaked on blood agar plates and incubated at 37° C for up to 3 hours in air added with 5% of CO_2.
hours.
The carbaNP and ESBL-NDP test were applied directly to positive blood culture in a lab routine setting, and reduced the reporting time to a 5 hours
Noteworthy, the same subculture can be used for Maldi-TOF identification and antimicrobial susceptibility test, too, thus allowing one to obtain a complete report in 24 hours



1.Nordmann P, Poirel L, Dortet L (2012) Rapid detection of carbapenemase-producing Enterobacteriaceae, Emerging infectious diseases
2.Nordmann P, Dortet L, Poirel l, Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae
3.. Dallenne *et al.* Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 2010

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