

Multiplex immunochromatographic detection of OXA-48, KPC and NDM carbapenemases: impact of the inoculum, antibiotics and agar

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Background

- Carbapenemase-producing Enterobacteriaceae (CPE) are an important threat to global public health
- OXA-48, KPC, VIM and NDM most important carbapenemases worldwide
- Detection of carbapenemases by immunochromatographic assays (ICT) has been recently established

Objectives

- Assessment of the new multiplex ICT RESIST-3 O.K.N. (Coris, Gembloux, Belgium), which detects OXA-48-like, KPC and NDM carbapenemases
- Evaluate performance when tested directly from susceptibility testing plates (Mueller Hinton agar [MHA])
- Analyze the impact of the inoculum and antibiotic disks on sensitivity and specificity

Methods

- Analysis of the ICT with 63 characterized, carbapenem resistant isolates
 - 51 carbapenemase producers, 12 carbapenemase negative
- The ICT was assessed using 5 different conditions from MHA
 - 1 µl or 10 µl inoculum harvested in the absence of antibiotic pressure
 - 1 µl taken from the inhibition zone of either ertapenem (EPM), imipenem (IPM) or meropenem (MER) disks

Results

- The sensitivity of the ICT was 100 % for OXA-48-like and KPC and 94.4 % for NDM with the 1 µl inoculum (Table 1); specificity was 100%
- When harvested adjacent to a carbapenem disk, the sensitivity increased to 100 % (Table 1) and the time to positivity decreased (Fig. 1B)
- Time to positivity was higher for NDM compared to OXA-48-like and KPC (Fig. 1A)
- With zinc-supplemented MHA both the sensitivity increased and the NDM band became visible faster (Fig. 2)

Table 1: Sensitivity of the ICT is dependent on test condition

Inoculum	Sensitivity		
	OXA-48 like	KPC	NDM
1 µl (no AB)	100 % (25/25)	100 % (10/10)	94.4 % (17/18)
10 µl (no AB)	100 % (25/25)	100 % (10/10)	100 % (18/18)
1 µl EPM	100 % (25/25)	100 % (10/10)	100 % (18/18)
1 µl IPM	100 % (25/25)	100 % (10/10)	100 % (18/18)
1 µl MER	100 % (25/25)	100 % (10/10)	100 % (18/18)

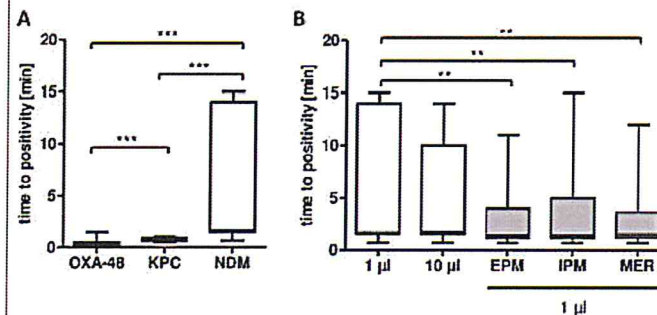


Figure 1

A: Differences in time to positivity between OXA-48-like, KPC and NDM-producing isolates for 1 µl inoculum
B: Impact of inoculum and antibiotics on time to positivity. 1 µl and 10 µl designate inoculum taken distant from antibiotic disks. Grey boxes indicate 1 µl inoculum that was harvested next to an antibiotic disk
EPM=ertapenem, IPM= imipenem, MER=meropenem
** = P < 0.01, *** = P < 0.001

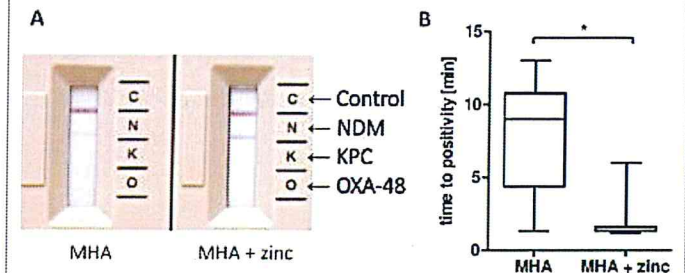


Figure 2

A: Effect of zinc sulfate on the intensity of the NDM band. Inoculum harvested from MHA (left) with a very faint band compared to MHA supplemented with 100 µl of a 10 mM ZnSO₄ solution (right).
B: Impact of zinc sulfate on time to positivity of NDM-isolates.

Conclusion

- The RESIST-3 O.K.N. ICT is a sensitive and rapid test for the detection of three highly prevalent carbapenemases. However, false-negative results for NDM can occur
- We recommend an inoculum of 1 µl that is harvested adjacent to an ertapenem or meropenem disk and the use of agars with sufficient zinc content to achieve the best performance
- The ICT is well-suited for clinical microbiology laboratories because of its simplicity and speed
- Before using the ICT under routine conditions for clinical isolates, we recommend to test several isolates (especially NDM producing) with the MHA used in the laboratory and to standardize the inoculum for this assay

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