Rapid naked-eye detection of carbapenemase-producing Enterobacterales from a positive hemoculture

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Abstract

Early detection of bloodstream infections caused by carbapenemase-producing Enterobacterales (CPE) is crucial for promptly optimising antimicrobial therapy and improving patient survival. Currently, confirming CPE relies on a modified carbapenem inactivation method (mCIM), which requires 24-hour incubation after a blood culture becomes positive, causing delays in adjusting treatment. Molecular testing of carbapenemase-producing genes is rapid but costly and not widely accessible in routine laboratories. While lateral flow immunoassays offer a quicker and cost-effective method that may overcome the above limitations. Here, we analysed 76 clinical carbapenem-resistant Enterobacterales (CRE) isolates from Ramathibodi Hospital. CPE was confirmed with mCIM and the carbapenemase-producing genes were simultaneously detected using the multiplex PCR-reverse hybridisation method. Evaluation of five common carbapenemase enzymes, including NDM, OXA-48-like, KPC, VIM, and IMP, was performed by the O.K.N.V.I. RESIST-5 lateral flow strip and compared with a gold standard mCIM and molecular assays. The strip detected all mCIM-positive strains (n=51), resulting in a sensitivity of 100%. Nevertheless, 4 of 25 mCIM-negative strains gave false positive results, causing a reduced specificity to 84%. Meanwhile, sensitivity and specificity were compared with a multiplex PCR-reverse hybridisation method, which was 98.15% and 90.90%, respectively. The O.K.N.V.I. RESIST-5 is rapid (20 minutes after a blood culture becomes positive) and easy to use. We observed excellent concordance with both mCIM (Kappa = 0.876) and molecular tests (Kappa = 0.903). Despite the decreased specificity, the O.K.N.V.I. RESIST-5 could serve as a screening tool for early detection of CPE in a positive hemoculture.

Introduction

Bloodstream infections (BSI) are a significant cause of morbidity and mortality worldwide. Traditionally, the identification of the causative pathogen relies on culture-based assays, which require pathogen growth on a solid medium before antimicrobial susceptibility testing can be performed. This process results in a prolonged turnaround time, delaying the initiation of appropriate treatment. Carbapenemase-producing Enterobacterales (CPE), a group of Gram-negative bacteria resistant to carbapenems, are particularly challenging to treat and are associated with poor clinical outcomes. Given the difficulties in managing infections caused by CPE, there is an urgent need for rapid diagnostic assays to detect CPE in BSI. Early identification of these pathogens is crucial for the timely optimization of antimicrobial therapy and for improving patient outcomes.

Objective

To test and evaluate the efficiency of the O.K.N.V.I. Resist-5 strip test in detecting Carbapenemase-producing Enterobaterales (CPE) by comparing with gold standard methods (mCIM) and molecular method (AMR Direct Flow Chip Kit).

Method

Sample Preparation

76 CRE isolates from clinical specimens at Ramathibodi Hospital (51 CPE and 25 non-CPE)

Suspend 5 colonies in 5 mL TSB ,incubate at 37°C with shacking

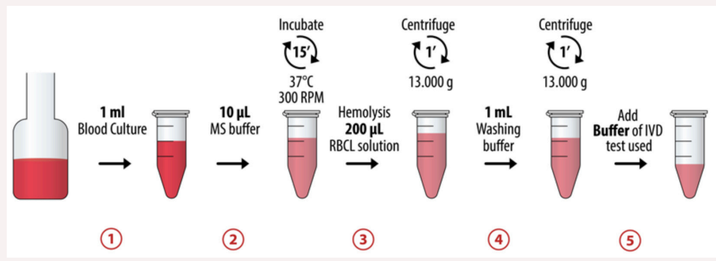
Measure cell suspension's turbidity

Dilute to 2.64×10^9 CFU/mL

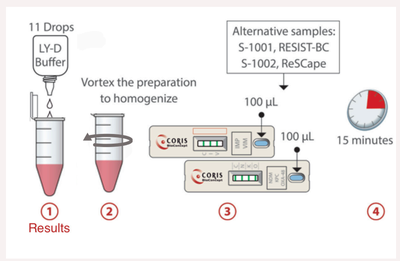
Inject 5 mL of solution into 30 mL negative hemoculture

Test Procedure

Treated the positive hemoculture with **RESIS-BC treated kit**



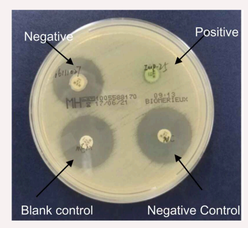
Read the result in **15 mins**



https://www.corisbio.com/products/oknvi-resist-5

Result analysis

Calculated the sensitivity, specificity, and kappa analysis of the O.K.N.V.I. Resist-5 strip test compared with gold standard methods (mCIM) and molecular method (AMR Direct Flow Chip Kit).



mCIM method

AMR Direct Flow Chip Kit

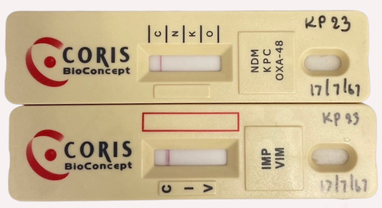
https://www.scienceopen.com/

Results

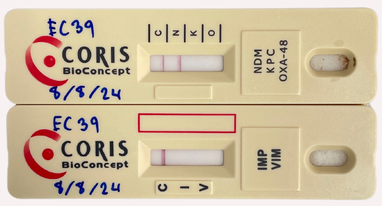
	mCIM positive	mCIM negative	PCR positive**	PCR negative
O.K.N.V.I. RESIST-5 positive*	51	4	53	2
O.K.N.V.I. RESIST-5 negative	0	21	1	20

mCIM Vs **O.K.N.V.I RESIST-5** : Sensitivity = 100%, Specificity = 84%, Kappa = 0.876

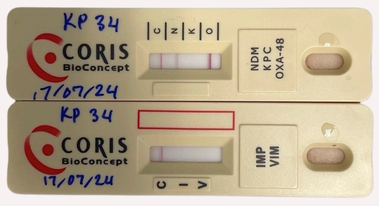
PCR Vs **O.K.N.V.I RESIST-5** : Sensitivity = 98.15%, Specificity = 90.90%, Kappa = 0.903



Negative



NDM



NDM&OXA48like

*Either NDM, OXA-48 like, KPC, VIM, or IMP enzymes detected

**Either NDM, OXA-48 like, KPC, VIM, or IMP genes detected

Conclusion

This study demonstrated the reliability of the O.K.N.V.I. RESIST-5 in rapid diagnosis of CPE bloodstream infections. The time-to-result of the O.K.N.V.I. RESIST-5 is less than 20 min, the mCIM is over 24 hrs, and the PCR assay is one hour. Given these advantages, the O.K.N.V.I. RESIST-5 can be implemented in the laboratory to detect CPE in blood cultures.