CARBAPENEM RESISTANCE - A GLOBAL CHALLENGE

Rising bacterial resistance to antibiotics has become a major public health issue worldwide. Although rarely reported a decade ago, carbapenem-resistant Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae*, are rapidly spreading worldwide, with the risk that they may evolve from multi-drug to pan-drug resistance. Carbapenem antibiotics are crucial for treating the life-threatening infections caused by these highly resistant Gram-negative bacteria. They are also “antibiotics of last resort” and it is essential to maintain their clinical efficacy.

Carbapenem resistance related to the production of a carbapenemase-type enzyme is currently the issue of most concern, since carbapenemase-producers are highly transferable.

Early identification of carbapenemase-producers in both infected patients and carriers is therefore critical to prevent the spread of infection and carbapenem resistance.

➔ Diagnosis of patients infected by carbapenemase-producing bacteria, but also screening of carriers, are first-line measures that contribute to:
  • Facilitate rapid implementation of infection prevention and control measures, e.g. patient isolation;
  • Prevent hospital-based outbreaks, and limit spread in the community;
  • Support epidemiological surveillance of the spread of carbapenemase producers at local, regional, national and global levels.

The use of chromogenic culture media, such as the CHROMID® range, followed by phenotypic confirmation with a rapid colorimetric test, RAPIDEC® CARBA NP, provides an innovative, easy-to-use and cost-effective strategy for the screening and confirmation of carbapenemase-producers.
CHROMID® CARBA
CHROMID® CARBA SMART
CHROMID® OXA-48

CHROMID® CARBA agar*, CHROMID® CARBA SMART agar and CHROMID® OXA-48 agar* are selective chromogenic media for the screening of Carbapenemase-Producing Enterobacteriaceae (CPE), and OXA-48 CPE, particularly KPC and NDM-1, in chronic carriers or "at risk" patients.

CPE are particularly multi-resistant bacteria that are capable of causing healthcare-associated infections (HAIs) and hospital epidemics. The detection of CPE carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of CHROMID® CARBA and CHROMID® OXA-48 media contributes to the active surveillance of CPE.

CHROMID® CARBA agar, CHROMID® CARBA SMART agar and CHROMID® OXA-48 agar (patents pending) consist of a rich nutritive base combining different peptones. The media contain:
• a mixture of antibiotics which enable the selective growth of CPE.
• three chromogenic substrates which enable the identification of the most frequently isolated CPE.

*see Instructions for Use at www.mybiomerieux.com for more information
## ARTICLES

**Evaluation of Multiple Methods for the Detection of Gastrointestinal Colonization of Carbapenem-Resistant Organisms from Rectal Swab**  
Simner P, et al.  
*Journal of Clinical Microbiology* 2016;54:1664-1667

**Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods.**  
Viau R, et al.  
*Clinical Microbiology Reviews* 2016;29:1-27

**Faecal carriage of carbapenemase-producing Gram-negative bacilli in hospital settings in southern France.**  
Pantel A, et al.  
*European Journal of Clinical Microbiology and Infectious Diseases* 2015;34:899–904

**Performance of different culture methods and of a commercial molecular assay for the detection of carbapenemase-producing Enterobacteriaceae in nursing homes and rehabilitation centers.**  
Sangman V, et al.  
*European Journal of Clinical Microbiology and Infectious Diseases* 2015;34:991–997

**Evaluation of a new chromogenic medium, CHROMID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey.**  
Zarikidou, et al.  
*European Journal of Clinical Microbiology and Infectious Diseases* 2015;34:519–525

**Evaluation of Five Chromogenic Agar Media and the Rosco Rapid Carb Screen Kit for Detection and Confirmation of Carbapenemase Production in Gram-Negative Bacilli.**  
Simner PJ, et al.  
*Journal of Clinical Microbiology* 2015;53:105-112

**Performance of CHROMID® CARBA Medium for Carbapenemases-Producing Enterobacteriaceae Detection during Rectal Screening**  
Papadimitriou-Olivgeris M, et al.  
*European Journal of Clinical Microbiology and Infectious Diseases* 2014;33:35-40

**Comparative evaluation of a novel chromogenic medium (CHROMID OXA-48) for detection of OXA-48 producing Enterobacteriaceae.**  
Giritch J, et al.  
*Diagnostic Microbiology and Infectious Disease* 2013;77:296–300

**Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media.**  
Day KM, et al.  
*Diagnostic Microbiology and Infectious Disease* 2013;75:187-91

**A comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae**  
Wilkinson KM, et al.  
*Journal of Clinical Microbiology* 2012;50:150-4

## POSTERS

**AMMI-CACMID 2016 / Vancouver (Canada)**  
Prospective Evaluation of bioMérieux’s CHROMID CARBA-SMART Agar Bi-Plate used with bioMérieux’s RAPIDEC CARBA-NP Assay for Rapid Phenotypic Detection of Carbapenemase-Producing Organisms (CPO) from Surveillance eSwabs  
Willey BM, et al.

**ECCMID 2016 / Amsterdam (The Netherlands)**  
Retrospective Evaluation of the Performance of the CHROMID CARBA SMART Bi-Plate to Detect Carbapenemase-Producing Organisms (CPO)  
Willey BM, et al.

**ECCMID 2015 / Copenhagen (Denmark)**  
Assessment on the Efficacy of CHROMID CARBA SMART Selective Chromogenic Media Bi-Plate (bioMérieux) for Detecting Carbapenem-Resistant Enterobacteriaceae  
Mendoza Jimenez T, et al.

**ECCMID 2013 / Berlin (Germany)**  
Evaluation of CHROMID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae  
Piazza A, et al.

First evaluation of CHROMID® OXA-48 agar - a new chromogenic medium for detection of Enterobacteriaceae-producing OXA-48 carbapenemase  
Dégagne L, et al.
Current methods for the detection of carbapenem-resistant organisms (CRO) include broth enrichment, direct selective culture, chromogenic media and detection of carbapenemase genes directly from rectal swabs. The study aimed to evaluate several of these methods for screening CRO from rectal swabs and determine the prevalence of gastrointestinal colonization with CRO among high-risk inpatient populations.

Five different methods for CRO detection were evaluated: the CDC broth enrichment method using etepenem for selection; a modified CDC broth enrichment method using etepenem and vancomycin for selection; a direct MacConkey plate with etepenem disk method; the CHROMID® CARBA agar plate method (new reformulated media*); the Check-Direct CPE Screen for BD MAX®. Two-hundred and thirteen frozen rectal ESwabs were collected from high-risk inpatients in a non-outbreak setting in a US hospital. After vortexing for 5 seconds, 100 μl of ESwab broth was inoculated into each of the media types and 25 μl was inoculated into a DNA sample buffer tube for the Check-Direct CPE Screen assay. All Gram-negative bacilli that grew within 27 mm of the etepenem disk or growth on CHROMID® CARBA were identified using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometry technology, and susceptibility testing was performed according to CLSI recommendations. Enterobacteriaceae resistant to one of the carbapenemases tested were evaluated to identify carbapenemase production by the Carba NP test. If positive, molecular genotyping was performed with Check-MDR.

The overall prevalence of colonization was determined to be 9.4% (N=20) for CRO and 2.3% (N=5) for carbapenemase-producing organisms (CPO). In conclusion, this method comparison showed that the Direct MacConkey plate method was the most sensitive test for CRO detection (95%) while CHROMID® CARBA and Check-Direct CPE Screen were the most sensitive for CPO detection (100%; all blaKPC). All methods had a specificity of >90% for CRO detection, and for CPO detection, the specificity ranged from 85-98% (84.6-90.4% for culture-based methods only). In this study, the CDC broth enrichment methods performed poorly compared to direct inoculation methods, negating the need for broth enrichment step.

“…the CHROMID CARBA media (100%; N=5; all blaKPC) was the most sensitive for CPO detection among culture based methods.”

KEY POINTS
- Among the culture-based methods, CHROMID® CARBA showed the best performance for screening of carbapenem-producing Enterobacteriaceae (CPE).
Faecal carriage of carbapenemase-producing Gram-negative bacilli in hospital settings in southern France

This is the first study to investigate the prevalence of faecal carriage of carbapenemase-producing Enterobacteriaceae (CPE) and carbapenemase-resistant Gram-negative bacilli (CR-GNB) in France. The prospective multi-center study took place in three University hospitals and four General Hospitals in the south of France during a non-outbreak period. A total of 1,135 faecal samples (1,074 stools and 61 rectal swabs) were screened with both chromogenic culture media (CHROMID® CARBA and CHROMID® OXA-48) and PCR (NUCLISENS easyMAG™ and NUCLISENS EasyQ® KPC). A collection of 202 characterized strains was used to validate the two chromogenic media, with inoculation of a low inoculum for OXA-48-producing Enterobacteriaceae (10^4 CFU) and a high inoculum (10^5 CFU) for all other strains. Plates were read at 18, 24 and 48 h of incubation.

Of the 1,135 samples, 27 (2.4%) carried CR-GNB isolates, with the identified species being P. aeruginosa (1.5%, n=17), Enterobacteriaceae (0.7%, n=8) and A. baumannii (0.2%, n=2). The sensitivity and specificity observed were 99% and 92% for CHROMID® OXA-48 and 69% and 95.5% for CHROMID® CARBA. Despite the low prevalence of faecal carriage of CPE in this study population, both media showed excellent sensitivity and the combination of CHROMID® CARBA and CHROMID® OXA-48 allowed 99% detection of CPE with 95% specificity at 18h.

This first French study showed very low dissemination of CP-GNB in hospitalized patients in southern France in a non-outbreak context. However, the increasing number of reports of epidemic cases in this area requires reinforced vigilance and control measures, including strict hand hygiene and screening of patients who may be at risk of CPE carriage, to prevent and limit the spread of these multidrug-resistant organisms.

“…the combined use of CHROMID® CARBA & CHROMID® OXA-48 should allow accurate detection of all clinically relevant carbapenemases.”

Performance of different culture methods and of a commercial molecular assay for the detection of carbapenemase-producing Enterobacteriaceae in nursing homes and rehabilitation centers

The study evaluated the hypothesis of a carbapenemase-producing Enterobacteriaceae (CPE) reservoir in a geriatric chronic care center. It also compared the performance of CHROMID® OXA-48, CHROMID® CARBA agars and MacConkey agar after enrichment broth followed by a molecular assay, Check-Direct CPE, for the screening of CPE to evaluate the intestinal carriage by rectal swabs. A total of 384 rectal swabs from 3 nursing homes and one rehabilitation center were collected using Eswab devices from COPAN. An amount of 100 µl was inoculated onto each of the 3 agars: CHROMID® CARBA, CHROMID® OXA-48 and on MacConkey with a temocillin/meropenem disk. In parallel, 100 µl were inoculated into an enrichment broth with ertapenem. After incubation for 4 hours at 35°C, 100 µl were inoculated onto MacConkey. Isolates were retrieved from 261 patients, and 257 showed growth on the MacConkey agar.

Two readings were performed after 24 and 48h of incubation. Identification of all colonies was performed using MALDI-TOF technology. Check-Direct CPE was performed on all Enterobacteriaceae isolates with meropenem MIC>8 µg/ml and/or temocillin MIC>165 µg/ml and each Eswab was analyzed by Check-Direct CPE for the detection of blad CARBA, blad OXA-48, blad NDM.

Only one of the 257 included residents/patients was a true asymptomatic carrier of CPE. Growth of K. pneumoniae was observed on this patient’s rectal screening culture after 24 h on CHROMID® OXA-48 and on MacConkey agar with and without ertapenem enrichment broth within the defined zone surrounding the temocillin/meropenem disk. The isolate gave a positive result with Check-Direct CPE directly on the Eswab, but was missed by CHROMID® CARBA. The use of an enrichment broth (CDC protocol) did not enhance the detection rate of CPE but increased the turnaround time of the analysis. The prolonged incubation of chromogenic media up to 48h did not increase the recovery rate of CPE. Use of MALDI-TOF rapidly confirms the species grown on the chromogenic media with or without characteristic colors and helps to save costs.

Since only one case of CPE OXA-48 was found, this survey could not confirm the presence of a CPE reservoir in nursing homes in Belgium. The specificity of the different methods was at least 97%. The use of the CHROMID® CARBA SMART bi-plate combining CHROMID® CARBA and CHROMID® OXA-48 to recover CHROMID® CARBA, CHROMID® OXA-48 and MacConkey agar after enrichment broth followed by a molecular assay, Check-Direct CPE, for the screening of CPE to evaluate the intestinal carriage by rectal swabs.

“…”In this study’s regions [in Belgium], where OXA-48 is a problem, the use of chromogenic biplates such as CHROMID® CARBA SMART (bioMérieux), combining CHROMID® CARBA and CHROMID® OXA-48 to recover both OXA-48, KPC and NDM is worthy of consideration.”
Evaluation of a new chromogenic medium, CHROMID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey

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(4) University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia.

This study evaluated a new chromogenic medium, CHROMID OXA-48, for the isolation of carbapenemase-producing Enterobacteriaceae (CPE) directly from rectal swabs from hospitalized patients. The performance of CHROMID OXA-48, as well as the CHROMID CARBA chromogenic medium, was compared to the broth enrichment method (5 ml TSB plus 30 µg ertapenem) recommended in the CDC protocol.

Screening of 302 hospitalized patients was performed by the three methods using rectal swabs. Thirty-three patients (11%) were found to be colonized with CPE. All CPE isolates were confirmed to be OXA-48 producers by both phenotypic testing and PCR. The dominant species, Klebsiella pneumoniae, was isolated from 31 patients. One patient was colonized with E. coli only, one with E. cloacae only and one with both K. pneumoniae and E. coli (all with OXA-48 carbapenemase).

Although CHROMID CARBA shows excellent performance with all other classes of CPE, it has limited efficiency in settings where OXA-48 is the dominant carbapenemase. In this study, the CHROMID OXA-48 medium proved to be highly useful for the detection of OXA-48 producing Enterobacteriaceae and was superior to the CDC broth-based method.

The combined use of these two complementary media demonstrated acceptable sensitivity (90.9%) and the highest specificity (98.5%) and enabled isolation of CPE within 18-20 hours.

In conclusion, combined screening using the CHROMID OXA-48 and CHROMID CARBA media proved to be the optimal solution for detection of Enterobacteriaceae with all commonly encountered carbapenemases.

“Overall, […] CHROMID CARBA was the most sensitive and specific chromogenic media evaluated for the detection of CPGNB…”

KEY POINTS
- CHROMID OXA-48 is highly useful for detection of OXA-48 producing Enterobacteriaceae and superior to the CDC protocol.
- When used in combination with CHROMID CARBA, CHROMID OXA-48 offers a highly effective solution for detection of CPE.
- CHROMID CARBA SMART combines both CHROMID CARBA and CHROMID OXA-48 media in a convenient bi-plate format.

Evaluation of Five Chromogenic Agar Media and the Rosco Rapid Carb Screen Kit for Detection and Confirmation of Carbapenemase Production in Gram-Negative Bacilli

Simner P1, Gilmour MW2, DeGagne P3, Nichol K4, Karlowsky JA5

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(b) University of Manitoba, Department of Medical Microbiology and Infectious Diseases, Winnipeg, Manitoba, Canada

This study set out to evaluate the analytical performance, cost and turnaround time of screening and confirmation methods, in order to determine the preferred workflow to detect carbapenemase-producing Gram-negative bacteria (CPGNB) in the clinical microbiology laboratory.

The first part of the study evaluated five different chromogenic media for CPGNB screening: Oxitell Brilliance ESBL, Oxitell Brilliance CRE, CHROMID CARBA, CHROMAgar Colores CST and CHROMAgar Colores KPC. Then a method comparison was performed between the modified Hodge test (MH) and the RCS test for confirmation of carbapenemase production. Finally, multiplex PCR was performed on all isolates.

A total of 150 isolates with 49 GNB harboring carbapenemases were tested. Among the three chromogenic media designed specifically for detection of CPGNB, CHROMID CARBA demonstrated the highest sensitivity and specificity, 89.8% and 95% respectively, followed by Colorex CRE 96.7% and Sp 92.1% and Brilliance CRE (Se 77.6% and Sp 87.1%). For the two confirmatory phenotypic tests, sensitivity was 75.5% and 98.0% and specificity was 93.1% and 100% for MH and RCS respectively. Convention PCR gave a sensitivity and specificity of 95.9% and 100% respectively.

By pairing the top-performing screening and confirmatory tests, the optimal workflow in terms of performance, cost and time was found to be CHROMID CARBA for screening, followed by the RCS test or PCR as the confirmatory method. This pairing gave a combined sensitivity of 89.9% and specificity of 100%. The main limitation of this algorithm is the poor detection of OXA-48 producers.

“…CHROMID CARBA was the most sensitive and specific chromogenic media for the detection of CPGNB…”

KEY POINTS
- CHROMID CARBA demonstrated the highest sensitivity and specificity for the detection of carbapenemase-producing gram-negative bacteria.
- CHROMID CARBA is recommended in this study as the first step in a CPGNB screening algorithm.
Rapid identification of patients colonized by carbapenemases-producing Enterobacteriaceae (CPE) is essential for implementation of infection control precautions. To implement preventive measures and control the spread of CPE, chromogenic CHROMID® CARBA medium was compared with two culture-based screening methods (CDC procedure and MacConkey agar with imipenem (MCI)) for its performance in detecting carbapenemase-producing Enterobacteriaceae (CPE) during a faecal screening surveillance program.

Double rectal swabs were collected from patients hospitalized in the ICU on admission and every 5-7 days during hospitalization. One swab was directly inoculated onto the solid media CHROMID® CARBA plate and MacConkey agar with imipenem, while the other was tested according to CDC protocol.

Suspected colonies from all procedures were identified to species level and tested for their susceptibility to carbapenems by phenotypic tests. All carbapenem non-susceptible isolates were tested by the Modified Hodge Test (MHT) and synergy tests. Positive results were confirmed by PCR testing for carbapenemase gene detection. The performance of all three procedures was statistically analyzed as compared to MHT and PCR results for the presence of carbapenemase-encoding genes.

Out of 177 rectal samples tested, 86 samples were found to contain one or more CPE: verified by molecular detection of carbapenemase-encoding genes among isolated Enterobacteriaceae. Sensitivity of CHROMID® CARBA and CDC methods was similarly high for CPE in clinical samples (96.5% and 98.8% respectively) compared to MCI (89.5%). CHROMID® CARBA had higher specificity before and after Gram staining (91.2% and 100% respectively) compared to the other two media (80.2% and 80.2% for CDC; 31.9% and 70.3% for MCI).

CHROMID® CARBA performed with high accuracy among the phenotypic methods applied, giving early results.

"... CHROMID® CARBA has demonstrated high accuracy when applied for CPE screening in rectal swabs [and] can be recommended for routine application in surveillance and infection control practices"
Prevalence and molecular characterization of *Enterobacteriaceae* producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media


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The aim of this study was to evaluate the performance of 2 chromogenic media (CHROMID CARBA and Brilliance CRE) recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE) in stool samples from patients attending a military hospital in Rawalpindi, Pakistan. Further aims included the identification of factors that might predispose to faecal carriage of CPE and to assess the prevalence and genotypic diversity of CPE in this population.

One hundred and seventy-five stool samples were collected from distinct patients attending the military hospital (143 on surgical wards and 32 outpatients). Of the 175 patients, 32 (18.3%) had faecal carriage of CPE and all produced NDM-1 carbapenemase. All of these 32 patients were detected using CHROMID CARBA compared with 20 patients (62.5%) detected using Brilliance CRE (P = 0.0015). If only colored colonies were considered as presumptive CPE, CHROMID CARBA also showed very high specificity (98%) with only 5 false-positive isolates of *Enterobacteriaceae* recovered from 175 samples.

In this study, duration of hospitalization and treatment with coamoxyclav were statistically associated with a higher likelihood of carriage of CPE (P ≤ 0.05). The majority of NDM-1-producing *Enterobacteriaceae* co-produced CTX-M-1 group extended spectrum β-lactamase (ESBL), and one third produced armA-type methylase. NDM-1 carbapenemase was most commonly found amongst commensal types of *Escherichia coli*, especially phylogenetic group B1.

“Thirty-two patients were detected with faecal carriage of NDM-1 […] and all of these patients were successfully detected using CHROMID® CARBA alone.”

**CHROMID® CARBA – CHROMID® CARBA SMART – CHROMID® OXA-48**

A comparison of four chromogenic culture media for carbapenemase-producing *Enterobacteriaceae*

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(b) Microbiology Department, Royal Hallamshire Hospital, Sheffield, United Kingdom
(c) School of Life Sciences, University of Northumbria, Newcastle upon Tyne, United Kingdom

The aim of this study was to evaluate the suitability of chromogenic media recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE).

Four chromogenic media and two selective broths were challenged with a collection of *Enterobacteriaceae* with well-defined β-lactamases and 100 stool samples.

With low inoculum of 130 isolates of CPE, the sensitivities of the four chromogenic media were: Brilliance CRE, 78%; CHROMID Carba, 91%; CHROMID ESBL, 96%; and Colorex KPC, 56%. The corresponding sensitivities of Trypticase soy broth plus ertapenem or meropenem were 78% and 47%, respectively.

CHROMID Carba showed optimal performance for both sensitivity (91%) and specificity (89%) for the detection of CPE with the collection of isolates used in this study.

“CHROMID® CARBA showed optimal performance with the collection of isolates used in this study […] Chromogenic media have the potential to provide useful tools for convenient and inexpensive screening of patients for CPE.”

**CHROMID® CARBA – CHROMID® CARBA SMART – CHROMID® OXA-48**

JOURNAL OF CLINICAL MICROBIOLOGY

2012;50:3102-4

**CHROMID® CARBA** is the most specific chromogenic medium for CPE screening with a high level of sensitivity.
Prospective Evaluation of bioMérieux’s CHROMID CARBA-SMART Agar Bi-Plate used with bioMérieux’s RAPIDEC CARBA-NP Assay for Rapid Phenotypic Detection of Carbapenemase-Producing Organisms (CPO) from Surveillance eSwabs

BM Willey, G Ricci, A Magalang, E Villena, DA Boyd, L Matave, P Lo, M Mulvey, T Mazzulli, SM Poutanen

Mount Sinai Hospital/LiU, Toronto, Toronto, Wilfred Older Health Sciences Centre, Brampton, National Microbiology Laboratory, Winnipeg, Canada

ABSTRACT

E. coli. In detecting CPO from surveillance swabs, laboratories considering significant (PS) growth from 40 (4.3%) swabs [170 (9.2%) / 24166/49 (8.1%); 18/19 (E. coli; dark blue), 40/76 (E. faecium, blue) were taken as CPO-positive, with the proviso that RAPIDEC-negatives, algorithm when RAPIDEC CARBA-NP was used directly from colonies for CPO detection from surveillance swabs. Outcomes were compared with final laboratory results only after completion of confirmation CPO testing. Sensitivities and specificities with 95% confidence intervals were calculated using www.graphpad.com.

METHODS

Consecutive eSwabs (typically rectal +/- nasal specimens) received by Consecutive eSwabs (typically rectal +/- nasal specimens) received by 1st half of study; lots 3 and 4 used during 2nd half were used during 1st half of study, lots 3 and 4 used during 2nd half

RESULTS

A total of 928 swabs grew CPO from both CHROMID® CARBA-SMART and RAPIDEC® CARBA-NP. Results from 4 lots by lot

INTRODUCTION

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RAPID accurate detection of pan-resist-resistant CPO is crucial for risk reduction. The potential for phenotypic testing to be used directly from CARBA-SMART chromogenic colonies with the understanding that RAPIDEC-negative isolates would require PCR to rule out CPO based on results from prior studies.

Methods: Consecutive rectal +/- nasal surveillance swabs (56% with known CPO/non-CPO content) were plated by WASP (3μL) to 3 places OXA agar (designed to be sensitive and specific for class D CPO). This study compared CPO detection from surveillance swabs used directly from CARBA-SMART chromogenic colonies with the understanding that RAPIDEC-negative isolates would require PCR to rule out CPO based on results from prior studies.

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INTRODUCTION

RAPID accurate detection of pan-resist-resistant CPO is crucial for risk reduction. The potential for phenotypic testing to be used directly from CARBA-SMART chromogenic colonies with the understanding that RAPIDEC-negative isolates would require PCR to rule out CPO based on results from prior studies.

Methods: Consecutive rectal +/- nasal surveillance swabs (56% with known CPO/non-CPO content) were plated by WASP (3μL) to 3 places OXA agar (designed to be sensitive and specific for class D CPO). This study compared CPO detection from surveillance swabs used directly from CARBA-SMART chromogenic colonies with the understanding that RAPIDEC-negative isolates would require PCR to rule out CPO based on results from prior studies.
Chromobacterium* Carbapenem SMART

Retrospective Evaluation of the Performance of the CHROMID CARBA-SMART Bi-Plate to Detect Carbapenemase-Producing Organisms (CPO)

Mount Sinai Hospital/CF, University of Toronto, McMaster Institute, Toronto; William Dakole Health Sciences Centre, Brampton; National Microbiology Laboratory, Winnipeg, Canada

ABSTRACT

Background: Optimum methods for detecting CPO from surveillance specimens have yet to be determined. Ideally, sensitivities of screening methods should be 100%, while accompanying specificities should be as high as possible to minimize confirmatory rule-out work. This retrospective study evaluated performance of bioMérieux’s CHROMID CARBA-SMART, a chromogenic biplate where CPO agar selects for all CPO and the OXA agar is designed to select for OXA48-type CPO only.

Methods: 259 species-diverse clinical isolates, highly-characterized by PCR/sequencing, were blinded to prevent bias. They included 221 CPO (108 class A: 99 KPC, 4 SME, 2 SME, 2 ES; 108 class B: 73 NDM, 6 VIM, 1 IMP; 26 class D: OXA, OXA48, OXA232; OXA244; 7 class B+D; NDM-OXA48, NDM-OXA232) and 38 non-CPO with mixed mechanisms (deterepressed-ampC, ESBL, Escherichia coli). Standard saline 0.5 MacFalden equivalent suspensions, prepared using colonies growing closest to enterobacteriaceae disc placed on MacConkey agar sub-cultures for selective pressure, were transferred to empted Copic® stubs for automated inoculation (10µl/side) to CARBA-SMART by the AWP system. After overnight (~4pm-~10am) incubation at 37°C, quantity, colour and size of colonies were documented independently by 5 readers. Consensus data were analyzed for sensitivity (Sn) and specificity (Sp) for 1) all CPO on CPO agar, 2) class CPO on OXA agar, and 3) overall CPO detection of both agar combinations. 95% confidence intervals (CI) were calculated using www.graphpad.com.

Results: Only 1 NDM+/F+ carbon and 1 OXA48+ E. coli were not detected by either agar on CARBA-SMART resulting in an overall Sn of 99.1% (96.9-99.7). The CPO agar grew all but 6/221 CPO (1 NDM- Proteus mirabilis and 5 OXA48-type Enterobacter cloacae) resulting in a CPO detection Se of 99.8% (97.3-99.9), and Sn by class was: A (100.0%, 99.5-100.0), B (98.8%, 92.9-99.9), and D (84.9%, 68.8-95.8). The OXA agar grew all but V53 class D (1 OXA48-E. coli that also failed on CABS) and also grew 1 class A (SM+ Sonattering marcescens) and 2 class B (1 NDM+ Acinetobacter baumannii, 1 V53+ Pseudomonas putida) CPO; corresponding Sn (99.0% CI) was 97.5% (83.4-99.9) for class D only and as expected it was low for any CPO (15.8% 11.6-21.3). Of the 38 non-CPO, 12 grew on CABS and 1 on OXA-agar, resulting in Sn (95% CI) of 69.4% (52.8-81.1) and 88.2% (91.1-95.5), respectively.

Conclusions: This evaluation of the CARBA-SMART chromogenic biplate found the CPO agar to complement the CHROMID CARBA at 4% OXA48-type CPO that were not detected on CABS grew on OXA, thus improving overall CPO detection (Sn) from 97.3% to 99.1%. These data support prospective evaluation.

INTRODUCTION

Rapid accurate detection of pan-resistant CPO is crucial for risk-reduction is patient care and to prevent outbreaks. Molecular testing is considered “gold standard” for confirming CPO, but no single assay yet detect all possible genotypes. CPO evolve rapidly with novel types appearing without warning, either types diversify via genetic drift at rates that have, in some instances, unknowingly reduced PCR detection sensitivities. Thus routine detection of CPO from surveillance swabs using PCR methods only, is prone to miss new genotypes in this rapidly changing environment. Even through PCR is an important tool for large laboratories to enable rapid CPO detection (<6h) direct from swabs in high-risk situations, albeit if only for the limited array of more common CPO genotypes, routine use of molecular assays for CPO detection is cost-prohibitive, especially in low-prevalence settings.

Thus for routine CPO surveillance, rectal swabs or other screening specimens first undergo selective culture. Past studies have found most “CPO-specific” screen agar to be poorly specific and lack sensitivity for certain CPO genotypes. Thus, for maximum sensitivity (the expense of specificity), many laboratories use MacConkey CV agar with a carbapenem disc on the main inoculum to select CPO from mixed flora, or use ESBL agar (MacConkey CV with 2mg/L cephalosporin), with the understanding that some rare CPO may be missed by the latter method if unaccompanied by ESBL genes (i.e. rare OXA48 isolates).

In order to increase CPO detection sensitivity of their CHROMID agar brand (CHROMID CABSM SMART was insensitive to OXA48, CPO biokemina created a biplate, the CHROMID CARBA-SMART (Figure 1)). This biplate consists of “OXA” agar side-by-side the original CHROMID agar, where OXA is claimed to have been formulated to specifically improve detection of class D CPO. This study evaluated the CARBA-SMART to determine whether there had been an overall improvement in CPO detection capabilities as a result of this modification of their product.
**RESULTS**

CARBA-SMART performed well in this study (Table 2). Together the agar detected most of the wide range of genetically diverse challenge CPO, as evidenced by an overall sensitivity (99.1%) of 99.1% (96.6-99.97) (Table 3). Only 1 Proteus mirabilis (bioMérieux) and 1 Escherichia coli (bioMérieux) were repeatedly missed by both agars.

Class D CPO agar grew all but 1/3 (1/3) CPO (NDM+ P- models and OXA48-like E. coli (NDM+ E. coli) resulting in a CPO detection sensitivity (99.1%) of 99.1-99.97). Sensitivity by class was: A: (100), B: (99.9%), C: (98.9%) and D: (84.9%-98.9%).

OXA agar grew all but 1/3 Class D CPO (OXA48-like E. coli that also failed on CARBA). It also grew 3 non-D CPO including 1 class A (brown-producing Serratia Marcescens) and 2 class B (1 NDM+ Acinetobacter baumannii, 1 VAP-Pseudomonas pseudo-typhus, possibly due to intrinsic mechanisms resistant to the proprietary antibiotic selective agent in OXA agar). Corresponding sensitivity (99.1%) for OXA was 97% (95.4-99.99) for class B only, and in expected, it was low for any CPO (15.8% [11.6-21.3]).

Of 38 non-CPO, 12 grew well on CARBA (all Intermediates) and 1 E. coli on OXA, resulting in specificities (99.1%) of 64.4% (52.5-81) and 98.2% (95.4-99.5), respectively (Tables 2 and 3).

**CHROMID® CARBA SMART**

Retrospective Evaluation of the Performance of the CHROMID CARBA-SMART Bi-Plate to Detect Carbapenemase-Producing Organisms (CPO)

**CONCLUSION & DISCUSSION**

Use of bioMérieux's CARBA-SMART as a CPO detection agar • This retrospective CARBA-SMART evaluation, which used highly-diverse and well-characterized CPO and non-CPO, found the combined use of CARBA and OXA agars to detect 99.1% of CPO overall while maintaining high specificities on OXA (98.2%).

Since 4 of 5 blaOXA48-like CPO that failed to grow on CARBA were easily detected on OXA agar, the CPO detection sensitivity of CARBA-SMART improved overall as compared to CHROMID CARBA agar alone [97.3% (93.7-99.9%) vs. 99.1% (96.6-99.97)] even though the detected improvement was not statistically significant (P=0.3).

While OXA agar was very specific, reproduce growth on CARBA agar of breakthrough non-CPO and growth on both OXA and CARBA agars of isolate-positive species, indicates that testing to eliminate oxazolidine-positive Gram-negative species and MALDI-TOF to rule out species with intrinsic resistance should precede or accompany testing of remaining Enterobacteriacae for CPO. • As CARBA-SMART is WASP compatible, and most CPO grew <10μL, and since its chemistries for differentiation of key species are in keeping with common chromogenic agars used for urine culture, it would be simple to introduce into routine laboratory workflows.
Assessment of the Efficacy of CHROMID CARBA SMART Selective Chromogenic Media Bi-Plate (bioMérieux) for Detecting Carbapenem-Resistant Enterobacteriaceae

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OBJECTIVE
Carbapenem-resistant Enterobacteriaceae (CRE) are multidrug-resistant bacteria that may be implicated in healthcare-associated infections. Detection of colonized patients and their subsequent isolation in single rooms is essential for prevention and control of nosocomial infections due to these microorganisms.

CHROMID® CARBA SMART selective chromogenic media bi-plate (bioMérieux) is an agar medium for rapid detection of CRE in direct sample culture. The aim of this study is to assess the efficacy of this medium for detecting CRE.

METHODS
This study was carried out at the Hospital Universitario de Canarias, a 600-bed tertiary care hospital in Tenerife, Spain from May 26th to July 1st, 2014.

Rectal and pharyngeal swabs were collected from every patient admitted in the Intensive Care Unit (ICU). We also collected rectal swabs from every patient admitted in 12 medical or surgical wards where a CRE had been previously detected in clinic samples from patients admitted to the facility, and each week up to two weeks after the last patient with CRE had been discharged, according to national and international guidelines.

Samples were cultured in CHROMID® CARBA SMART selective chromogenic media biplate (bioMérieux) and those from patients admitted in ICU were cultured on McConkey agar (bioMérieux). All the CRE detected were classified as OXA-48-type carbapenemase-producing Enterobacteriaceae.

RESULTS
We analyzed 562 samples from 148 patients. 163 of the samples were from patients admitted in ICU. We detected 23 samples with CRE, 21 in rectal swabs and 2 in a pharyngeal swab. Total prevalence: 3.91%. Prevalence by wards: General Surgery 13% (5), Neurosurgery 13.5% (6), Internal Medicine 6.2% (8), Oncology 3.1% (3), ICU 2.4% (4).

In every case in which CHROMID® CARBA SMART was positive, it was confirmed as CRE (100% sensitivity). Regarding patients admitted in ICU, McConkey culture did not grow CRE in any of the negative cases in CHROMID® CARBA SMART (100% specificity) and the four positive cases of CRE grew both in CHROMID® CARBA SMART and in McConkey agar (bioMérieux). All the CRE detected were classified as OXA-48-type carbapenemase-producing Enterobacteriaceae.

CONCLUSION
CHROMID® CARBA SMART selective chromogenic media bi-plate (bioMérieux) allows the rapid identification of CRE with 100% sensitivity and 100% specificity and it is useful for the early identification of carriers, saving microbiological costs.

CHROMID® CARBA

Evaluation of CHROMID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae

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INTRODUCTION AND PURPOSE
Carbapenems are used as a last-resort antibiotic class for the treatment of infections due to multidrug-resistant Enterobacteriaceae. However, during the last decade, carbapenem resistance has been increasingly reported and carbapenemase-producing Enterobacteriaceae (CRE) are emerging as a growing challenge in health care facilities. The clinically significant carbapenemases in Enterobacteriaceae belong mostly to: Ambler class A (KPC), the zinc-dependent class B (NDM, VIM, and IMP) and the class D (OXA-48-like) of β-lactamases. Carbapenemase-producing pathogens have been associated with high rates of morbidity and mortality particularly among critically ill patients with prolonged hospitalization. It is also noted that the carbapenemase genes harbored by CRE are mostly transposable and/or integron-encoded determinants that can easily disseminate to other enterobacterial strains and species.

Detection of CRE carriers, by selective chromogenic medium, is a useful tool for rapid and inexpensive screening of patients for CRE and is also available for daily use in many laboratories. Culture techniques for screening CRE have been tested, including methods that use in-house-prepared selective media, such as TSB containing a 10 μg carbapenem disk or selective chromogenic agar media, like CHROMID CARBA agar.

CHROMID CARBA agar is designed for CPE detection and is supplemented with a mixture of antibiotics that inhibit the growth of Gram-positive and non-CPE (4) and with three chromogenic substrates that may contribute to the recognition of enterobacterial species: Escherichia coli produce pink to burgundy colonies or translucent colonies with a pink to burgundy center, while Klebsiella, Enterobacter, Serratia, Citrobacter (KESC group) species produce blueish-gray to bluish-grey colonies.

The aim of this study was to evaluate the performance of CHROMID CARBA agar medium, provided by bioMérieux (Marcy l’Etoile, France) for: 1) detection and differentiation of a previously well-characterized collection of CRE with various enzymatic resistance mechanisms and 2) screening for CPE carriers.

METHODS
Overall 60 CRE (42 KPC, 11 VIM-type; 1 OXA-48 and 1 NDM-1) isolates and, as negative control, 40 non-CPE susceptible to carbapenems or resistant with mechanism other than carbapenemase production (24 CTX-M-type, 6 TEM-type, 6 CMY-16, 2 porin loss plus ESBL or AmpC, 5 hyper-producing isolates) were plated onto CHROMID® CARBA plate medium. Two different inoculum sizes (10 CFU/μl and 100 CFU/μl) of each isolate were plated onto the medium. All plates were incubated at 37°C and inspected for growth and colony colour after 18, 24, and 48 h. To validate the method of inoculum preparation, 10 strains were selected at random, 1 μl of the diluted suspension was inoculated onto each of three Columbia blood agar plates, and after incubation for 24 h at 37°C, the average number of colonies for each isolate was recorded.

The performance of CARBA agar medium was compared with the screening method recommended by CDC (5). Each of the suspensions described above was further diluted 1/20 in saline, and 100 μl of each was used to inoculate 5 ml of TSB containing a 10 μg imipenem disc and 5 ml of TSB containing a 1 μg meropenem disc. The broth-was incubated for 18 h at 37°C, and 100 μl of broth was then cultured onto MacConkey agar, which was incubated for 18 h at 38°C. The medium was assessed for its ability to inhibit the growth of other commensal non-CPE microorganisms in mixed cultures. Mixed cultures were prepared in different rates of CPE/non-CPE (1:100; 1:1000; 1:10000) in saline. 1 μl of the suspension was inoculated onto CHROMID® CARBA agar. All plates were incubated at 37°C and the average number of colonies was recorded after 24 h. Four hundred rectal swabs from laboratory routine were directly plated onto CHROMID® CARBA agar medium in two different Italian laboratories. The results were compared to standard routine laboratory method for CPE screening. All different colonies recovered from CHROMID® CARBA agar were subjected to identification with MALDI-TOF (VITEK MS, bioMérieux).
Evaluation of CHROMID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae

CONCLUSIONS

CHROMID® CARBA agar medium demonstrated good performance with the collection of CPE used, and also for CPE difficult to detect due to the low carbapenem MICs, such as OXA-48 and VIM-type producing strains. Extended incubation for 48h had impact on the specificity of CHROMID CARBA agar medium as two carbapenem resistant porin loss isolates grew after incubation for up to 24h (Fig. 2).

CHROMID® CARBA agar medium showed a high correlation with the routine procedures and in 2 cases a higher sensitivity in the detection of CPE. Further, this method allows the isolation of CPE strains from mixed cultures including at low concentrations.

RESULTS

All CPE strains grew on CHROMID® CARBA agar medium after 18h of incubation, independently of inoculum size, and developed characteristic coloration of species (Fig. 1). No growth was detected for control non-CPE isolates except for the two carbapenem resistant porin loss isolates that grew after 24h of incubation (Fig. 2). Colony counts, performed on 10 isolates, to validate the method of inoculum preparation revealed an average count of approximately 10^5 CFU/spot and 10^3 CFU/spot for high and low inoculum, respectively.

Out of the 400 rectal swabs analysed, 32 were positive with both the routine methods and CHROMID® CARBA agar methods and 2 were positive only for the latter. These two isolates were from patients with a low level of colonization (<4 colonies) and were confirmed as KPC producers with molecular methods. In 11 cases a growth of non-Enterobacteriaceae organisms was detected (2 Staphylococcus spp., 10 Pseudomonas spp., 1 Stenotrophomonas maltophila and 1 Enterococcus faecalis).

CHROMID® CARBA agar medium showed high sensitivity allowing to isolate CPE strains from mixed cultures also in minority concentration (Fig. 3).

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KEY POINTS

- CHROMID® OXA-48 allows screening of OXA-48 CPE.
- The association of CHROMID® CARBA and CHROMID® OXA-48 in this study allowed screening of all relevant carbapenemases (CPE).

**DISCUSSION**

**Detection of OXACPE:** All tested strains were detected after 18 hours of incubation on the CHROMID® OXA-48 medium. The sensitivity of this medium is excellent despite the low inoculum used (1.5 $10^3$ CFU/plate).

The specificity of detection of CHROMID® OXA-48 is also very high as 98% of the non-OXACPE tested were negative: the 2 false-positive strains growing on the medium were: (i) *S. marcescens* producing an IMP carbapenemase, and (ii) one VRE (*E. faecium*) that could easily be differentiated from EB by morphology, colonies coloration and/or Gram staining.

**Detection of all CPE:** The combination of CHROMID® OXA-48 and CHROMID® CARBA allows the detection of 99% of CPE with 95% of specificity after 18 hours. The false positive results are due to the growth of VRE on CHROMID® CARBA (negative after Gram staining) and of some strains resistant to carbapenem by impermeability. In contrast, only 61% and 69% CPE were detected after 18 hours of incubation by Brilliance CRE and Colorex KPC respectively. Most of the false positives on Brilliance CRE and Colores KPC are due to a lack of selectivity of the 2 media regarding EB strains with porin loss or producing HI.

**Clinical samples:** Those results were confirmed by the clinical study. Despite the low prevalence of digestive carriage of OXACPE in the south of France (0.3% in this study), the two media showed excellent specificities, 98.7% for CHROMID® OXA-48 and 95.5% for CHROMID® CARBA medium.

**CONCLUSION**

These studies highlight the high sensitivity and specificity of the CHROMID® OXA-48 medium for the detection of strains producing OXA-48 carbapenemase. The combination of the ready-to-use media CHROMID® CARBA and CHROMID® OXA-48 is the relevant solution which allows the optimal detection of all Enterobacteriaceae-producing carbapenemases, including OXA-48 carbapenemases, after only 18 hours of incubation. This combination of CHROMID® CARBA and CHROMID® OXA-48 should facilitate infection control and the prevention of epidemics, even in emerging countries.

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**Table 2:** Number of internal collection strains detected as positive on the different media

<table>
<thead>
<tr>
<th>Combination</th>
<th>CHROMID® OXA-48</th>
<th>CHROMID® CARBA</th>
<th>Colores KPC</th>
<th>Brilliance CRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nb of strains</td>
<td>18h</td>
<td>24h</td>
<td>18h</td>
<td>24h</td>
</tr>
<tr>
<td>37 OXA-48</td>
<td>37</td>
<td>37</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>35 Other carbapenemase</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>36 H. influenzae, ESBL</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>14 resistant by impermeability</td>
<td>14</td>
<td>14</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>10 Other Enterobacteria (OXA-23)</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>56 wild type</td>
<td>56</td>
<td>56</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>14 VRE</td>
<td>14</td>
<td>14</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>202 total</td>
<td>202</td>
<td>202</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 3:** Performance of the different chromogenic media solution for CPE detection

<table>
<thead>
<tr>
<th>Combination</th>
<th>CHROMID® OXA-48</th>
<th>CHROMID® CARBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity in %</td>
<td>61</td>
<td>69</td>
</tr>
<tr>
<td>Fertility in %</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity in %</td>
<td>95</td>
<td>93</td>
</tr>
</tbody>
</table>

**Table 4:** Clinical samples: growth and PCR results

<table>
<thead>
<tr>
<th>Combination</th>
<th>CHROMID® OXA-48</th>
<th>CHROMID® CARBA</th>
<th>PCR OXA-48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nb of strains</td>
<td>18h</td>
<td>24h</td>
<td>18h</td>
</tr>
<tr>
<td>115 clinical stools or rectal swabs</td>
<td>115</td>
<td>115</td>
<td>3</td>
</tr>
<tr>
<td>OXA-48</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other carbapenemase</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa wild type</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>P. aeruginosa OprD loss</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa OprD loss + Efflux</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>H. influenzae, ESBL and porin loss</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Colourless colonies</td>
<td>14</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

**Figure 3:** Distribution of the origin of the clinical specimens.

- Pediatric
- General medicine
- Geriatrics
- Intensive care
- Surgery
- Emergency
- Physiotherapy

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**Figure 2:** Distribution of the origin of the clinical specimens.

- Enterobacteriaceae: producing carbapenemases, including OXA-48 carbapenemases, after only 18 hours of incubation. This combination of CHROMID® CARBA and CHROMID® OXA-48 should facilitate infection control and the prevention of epidemics, even in emerging countries.
The RAPIDEC® CARBA NP test* consists of a ready-to-use strip for the rapid detection of carbapenemase activity in Gram-negative bacteria, such as Enterobacteriaceae, *P. aeruginosa* and in *A. baumannii*, using bacteria cultured on an agar medium.

The test is based on detection of hydrolysis of the ß-lactam ring of a carbapenem (imipenem). Hydrolysis acidifies the medium, changing the color of the pH indicator (phenol red solution). The color change is visible to the naked eye; no reading device is required. A color change within 2 hours indicates the presence of carbapenemase-producing activity.

The RAPIDEC® CARBA NP test provides detection of carbapenem resistance in 2 hours (compared with 24-48 hours using conventional tests). It can be performed directly on isolated colonies grown on recommended selective or non-selective agars.

The test is recommended for rapid detection of any carbapenemase activity of Enterobacteriaceae, and specifically the types most commonly found worldwide today: *Klebsiella pneumoniae* carbapenemase (KPC); New Delhi metallo-ß-lactamase (NDM); Verona integron-encoded metallo-ß-lactamase (VIM), imipenemase (IMP) and oxacillinase-48 (OXA-48). For example, KPC-producing bacteria can be detected in 30 minutes.

The test has excellent sensitivity and specificity and enables any laboratory to rapidly implement its own screening program for carbapenemase-producing bacteria. The test does not require any specific equipment or additional technology.

*see Instructions for Use at www.mybiomerieux.com for more information
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Kabir MH, et al.
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Poirel L, et al.

Evaluation of the RAPIDEC® CARBA NP test for the detection of carbapenemases in Gram-negative bacteria.
Lazareva I, et al.
ECCMID 2016 / Amsterdam (The Netherlands)

Use of the RAPIDEC® CARBA NP test (BioMérieux) for the detection of carbapenemase-producing Gram-negatives directly from positive blood cultures
Vourli S, et al.
ICAAC 2015 / San Diego (USA)

RAPIDEC® CARBA NP

The objective of the study was to evaluate the RAPIDEC® CARBA NP, a colorimetric test for rapid detection of carbapenemases, at two sites: Karolinska University Laboratory and Public Health England’s national reference laboratory.

A panel of 138 bacterial isolates previously characterized as positive for class A, B and/or D carbapenemase genes and 138 non-carbapenemase producers were tested with RAPIDEC® CARBA NP. Two carbapenemase-producing isolates carried both NDM and OXA-48-like genes. Molecular detection of the expected carbapenemase gene(s) was used as the reference method, and was performed by conventional and real-time PCR in-house assays.

RAPIDEC® CARBA NP detected 135 of 138 carbapenemase producers; 1 OXA-48-producing Klebsiella pneumoniae and 2 Acinetobacter baumannii producing OXA-23 and OXA-24 were not detected. Among ‘negative’ controls, 135 of 138 isolates were negative by RAPIDEC® CARBA NP. The exceptions were 1 Klebsiella oxytoca, which was later found to produce GES-5 carbapenemase, 1 Pseudomonas aeruginosa with OprD loss and increased efflux, and 1 Enterobacter cloacae with impermeability. When numbers were adjusted for the GES-5 producer, the overall sensitivity of the RAPIDEC® CARBA NP test was 97.8% and its specificity was 98.5%.

This study concluded that the RAPIDEC® CARBA NP test is an easy-to-use rapid test, taking less than 2.5 h for the detection of carbapenemase production and does not require specific equipment. It is a simple, relatively inexpensive method, making it feasible to be carried out by rather inexperienced technicians and in medium-income settings.

“The [RAPIDEC® CARBA NP] assay took less than two and a half hours to carry out, was user-friendly, and had a high overall performance, making it an attractive option for clinical laboratories.”

KEY POINTS

- The RAPIDEC® CARBA NP is simple and rapid test, requiring no specific equipment.
- The method showed very good performance (sensitivity: 97.8% and specificity: 98.5%).
- With a < 2.5 h turnaround time, the test could play an important role in preventing the spread of outbreaks caused by carbapenemase-producing Gram-negative bacteria, by leading to more rapid prevention and control measures.
Evaluation of the RAPIDEC CARBA NP test kit for detection of Carbapenemase Producing Gram Negative Bacteria

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(c) Department of Microbiology, Assam University, Silchar, India

The aim of this study was to evaluate the efficacy of the RAPIDEC® CARBA NP test in India.

A panel of 100 bacterial isolates were selected for testing:
• 50 contained different classes of carbapenemases (NDM, KPC, VIM, IMP, and OXA-48, all confirmed by PCR and sequence analysis)
• 50 were non-carbapenemase producers, of which 25 were carbapenem-sensitive and 25 were resistant to at least one of the carbapenems tested.

The RAPIDEC® CARBA NP test detected carbapenemase activity in 46/50 (92%) of the PCR confirmed strains. Carbapenemase activity was not detected in 3 OXA-48-positive strains (two Escherichia coli isolates [nonclonal] and one Klebsiella pneumoniae isolate), and one IMP-positive Escherichia coli strain.

Two carbapenemase negative carbapenem-resistant strains (Escherichia coli, Pseudomonas aeruginosa) gave false-positive results, but none of the carbapenem-sensitive bacteria were positive. Overall, the kit showed good efficacy with a high level of sensitivity (92.6%), specificity (96.2%) as well as positive and negative predictive values of 95.83% and 92.6% respectively.

“The [RAPIDEC® CARBA NP] assay took less than two and a half hours to carry out, was user-friendly, and had a high overall performance, making it an attractive option for clinical laboratories.”

KEY POINTS

- The RAPIDEC® CARBA NP test is simple, easy-to-perform and interpret, with high sensitivity and specificity, giving results within 30 minutes in most cases.
- The test provides a practical, relatively inexpensive solution for detection of carbapenemase-producing, multidrug-resistant Gram-negative bacteria in resource-limited settings.
- It should contribute to implementing infection prevention and control measures, e.g., patient isolation, to limit the spread of carbapenemase producers and help avoid outbreaks in healthcare settings.

Evaluation of the RAPIDEC CARBA NP, the Rapid CARB Screen and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae

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(3) Department of Bacteriology-Parasitology-Hygiene, Hôpital de Bicêtre, Assistance Publique – Hôpitaux de Paris, Le Kremlin-Bicêtre, France
(4) Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
(5) Hôpital Fribourgeois-Hôpital Cantonal, Fribourg, Switzerland

The aim of this study was to evaluate the performance of two commercial biochemical tests - RAPIDEC® CARBA NP and Rapid CARB Screen® (Rosco Diagnostica) - for the rapid detection of carbapenemase-producing Enterobacteriaceae compared with a home-made technique, the Carba NP test.

A panel of 150 enterobacterial isolates, including 132 isolates with decreased susceptibility to at least one carbapenem molecule, were tested for carbapenemase activity. The panel included 55 non-carbapenemase producers, 21 KPC producers, 21 NDM producers, 17 VIM producers, 11 IMP producers, 16 OXA-48 producers and 9 OXA-48-like producers (OXA-162, OXA-181, OXA-204, OXA-232 and OXA-244).

RAPIDEC® CARBA NP detected all carbapenemase producers except a single OXA-244 producer. Rapid CARB Screen® gave equivocal results for one KPC-2, two NDM-1, one OXA-48, five OXA-48 variant producers and did not detect one OXA-244 producer. The Carba NP test did not detect the same OXA-244 producer and gave equivocal results for one OXA-181 producer and one OXA-244 producer. Sensitivity and specificity were 99% (95% CI 94.3%-99.8%) and 100% (95% CI 93.5%-100%), respectively, for the RAPIDEC® CARBA NP test, 89.5% (95% CI 81.7%-94.2%) and 70.9% (95% CI 57.9%-81.2%) for the Rapid CARB Screen® and 94.3%-99.8%) and 100% (95% CI 93.5%-100%) for the Carba NP test.

RAPIDEC® CARBA NP showed the best performance compared to the Rapid CARB Screen for detecting any type of CPE (known and unknown carbapenemases). It is a rapid and easy-to-use diagnostic test for controlling the spread of CPE by detecting any kind of carbapenemase activity, and could be used for first-line screening of CPE in clinical settings.

In addition, the impact of using an adequate bacterial inoculum to obtain optimal performance with the RAPIDEC® CARBA NP test was specifically noted in this study.

“The RAPIDEC® CARBA NP is more specific and sensitive than the Rapid CARB Screen® for detecting any type of CPE (known and unknown carbapenemases).”

KEY POINTS

- The RAPIDEC® CARBA NP test was more specific and more sensitive than the Rapid CARB Screen® test in this study.
- It is rapid and easy to use for the detection of carbapenemase activity and can be used as a first-line CPE screening test in clinical settings.
Evaluation of the RAPIDEC CARBA NP test for the detection of Carbapenemases in Enterobacteriaceae

This study evaluated the performance of the RAPIDEC® CARBA NP test on a wide spectrum of beta-lactamase producing Enterobacteriaceae clinical isolates.

In total, 252 clinical isolates were included in the study. The study group comprised of 51/252 (20.2%) genetically confirmed carbapenemase producers (VIM, KPC, IMI, NDM, GIM, OXA-48). The negative control group comprised of 201/252 (79.8%) isolates, of which 152/252 (60.3%) were suspected carbapenemase producers, but found to be genetically negative for carbapenemase genes, and 49/252 (19.4%) were not suspected to be carbapenemase producers. All microorganisms were characterized phenotypically (susceptibility testing) and genetically.

Test reactions were read after incubation for 30 and 120 minutes. In total, 51 carbapenemase genes were detected in 252 Enterobacteriaceae isolates (20.2%): 13 blaKPC, 1 blaIMI, 1 blaIMP, 6 blaVIM, 10 blaNDM, 1 blaGIM, and 10 blaOXO-48-like. AmpC beta-lactamases were detected in 136 (54.0%) of the isolates and 101 isolates (40.0%) carried an ESBL.

After 120 minutes, sensitivity was 90.2%, specificity 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 97.6%. Reading after 30 minutes showed lower performance, with 27/51 carbapenemase producers giving a negative reading. Nineteen of these 27 isolates were blaOXO-48-like enzymes, underlining the relevance of reading the test after 120 minutes, particularly in case of blaOXA-48 suspicion.* Of the 27 isolates, 22 gave a positive result after 120 minutes of incubation.

The RAPIDEC® CARBA NP test showed very good performance and was demonstrated to be useful for reliable confirmation of carbapenemase-producing Enterobacteriaceae.

* Authors’ recommend incubating for 2 hours when OXA-48 carbapenemases are suspected, if there is a high-prevalence epidemiological context.

“The RAPIDEC CARBA NP test is a useful tool for the reliable confirmation of carbapenemase-producing Enterobacteriaceae”

RAPIDEC® CARBA NP

JOURNAL OF CLINICAL MICROBIOLOGY
2015; 53:3828-3832

RAPIDEC® CARBA NP test for rapid detection of carbapenemase producers

The objective of the study was to evaluate the performance of RAPIDEC® CARBA NP for detection of all types of carbapenemases in Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa.

Test performance was evaluated by testing 176 strains from various clinical origins (blood culture, urine, sputum, gut flora), of worldwide origin, isolated from 2010 to 2014. All types of carbapenemases in Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa were tested (frequently acquired, as well as rare carbapenemases).

The study included a total of 98 isolates producing all types of carbapenem-hydrolyzing beta-lactamases (VIM, KPC, IMI, NDM, GIM, OXA-48) and 75 carbapenemase-negative strains, of which, 52 were carbapenem-susceptible and 23 carbapenem-resistant (permeability defects).

All microorganisms were compared to Carba NP and characterized by molecular biology and MIC were defined with Etest methods according to US CLSI guidelines as updated in 2014.

In less than 2 hours after sample preparation, RAPIDEC® CARBA NP showed a sensitivity and specificity of 96%. This ready-to-use test is well adapted to the daily detection of carbapenemase producers in any laboratory worldwide.

“The use of the RAPIDEC® CARBA NP test may contribute to the identification of carbapenemase producers and improve infection control”

KEY POINTS

- The ready-to-use RAPIDEC® CARBA NP test is well adapted for routine detection of carbapenemase producers in any laboratory worldwide.
- Excellent sensitivity and specificity (96%) for reliable detection of carbapenemases in clinically significant Gram-negative bacteria.
Evaluation of the RAPIDEC® CARBA NP test for the detection of carbapenemases in Gram-negative bacteria

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2) North Western Medical University named after I. Mechnikov, Saint Petersburg, Russia

RESULTS

The results of the RAPIDEC® CARBA NP test with Quality Control strains were successful. The results of the RAPIDEC® CARBA NP test with carbapenemase-producing isolates are presented in Table 1 below. All of the NDM-, KPC-, and VIM-producing isolates and 7 of 9 VIM-producing isolates gave positive results already after 30 min of incubation. All isolates, producing OXA-type carbapenemases and 2 isolates, producing VIM-type carbapenemases, demonstrated negative results after 30 min of incubation and positive results after 120 min (see picture below). The sensitivity of the RAPIDEC® CARBA NP test after 30 min and 120 min of incubation was 65.4% and 100.0%, respectively. The specificity of the RAPIDEC® CARBA NP test after both 30 min and 120 min of incubation was 100.0%

Table 1

<table>
<thead>
<tr>
<th>Carbapenemases</th>
<th>Species</th>
<th>MIC (mg/l) range</th>
<th>Results at 30'</th>
<th>Results at 120'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P100</td>
<td>P100</td>
</tr>
<tr>
<td>OXA-48</td>
<td>Enterobacteriaceae (11)</td>
<td>1-2.5-5</td>
<td>2-16</td>
<td>0</td>
</tr>
<tr>
<td>NDM-1</td>
<td>Enterobacteriaceae (13)</td>
<td>8-64</td>
<td>8-64</td>
<td>15</td>
</tr>
<tr>
<td>KPC-2</td>
<td>Enterobacteriaceae (10)</td>
<td>8-64</td>
<td>8-64</td>
<td>10</td>
</tr>
<tr>
<td>VIM-type</td>
<td>Enterobacteriaceae (2)</td>
<td>1-4</td>
<td>0.5-16</td>
<td>7</td>
</tr>
<tr>
<td>OXA-48, OXA-23</td>
<td>A. baumannii (2)</td>
<td>2-16</td>
<td>2-16</td>
<td>8</td>
</tr>
<tr>
<td>None</td>
<td>A. baumannii (10)</td>
<td>0.0006-0.25</td>
<td>0.0006-0.25</td>
<td>0</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The RAPIDEC® CARBA NP test is suitable for rapid and easy evaluation of carbapenemase activity (OXA-type and VIM-type) might be slow, the manufacturer recommends a second reading after 2 hours incubation which increases the sensitivity up to 100% otherwise there is a risk of false-negative results with OXA-type and VIM-type carbapenemases producers.

KEY POINTS

- RAPIDEC® CARBA NP showed 100% sensitivity and 100% specificity for detection of carbapenemase-producing Gram-negative bacteria after incubation for 120 minutes in this study.

Use of the RAPIDEC® CARBA NP test (bioMérieux) for the detection of carbapenemase-producing Gram-negatives directly from positive blood cultures

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2. Department of Antimicrobial Agents, Chemotherapy, 2011, 53 (9), 2559-2566

RESULTS

Early detection of carbapenemase-producing (CP) gram-negative pathogens can be crucial for the choice of effective therapy and the outcome of bloodstream infections. Using conventional methods (culture, identification, susceptibility testing of the pathogen and phenotypic detection of carbapenemases), results are usually available 48-72hrs after blood culture positivity. On the other hand, rapid molecular detection of carbapenemases requires specific equipment and expertise.

The RAPIDEC® CARBA NP test (bioMérieux) is a rapid test for the detection of carbapenemase genes from pure cultures of Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa. Based on a biochemical method [1], in order to expand its clinical utility, we applied this test directly to positive blood culture broth.

**Table 1.** Performance of the RAPIDEC® CARBA NP test for the detection of carbapenemase-producing (CP) gram-negative bacilli isolated from blood cultures.

<table>
<thead>
<tr>
<th>CP-organism</th>
<th>A. baumannii</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>A. baumannii</td>
<td>A. baumannii</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**METHODS**

- **Isolation of BACTEC** Plus Anaerobic Culture Vials (BD) with 10ml of sterile human blood and 150FU of each isolate.
- **Thirty CP clinical isolates (KPC-3 and 2 VIM-producing K. pneumoniae, 17 OXA-type producing A. baumannii, and 3 VIM-type producing P. aeruginosa) and 4 negative controls (E. coli ATCC 25922 and three VIM-type clinical isolates).**
- **Incubation in BACTEC 900 system (BD) until positivity.**
- **Centrifugation of 6 ml broth samples of each positive bottle at 1600xg for 5 min. Contribution of supernatant at 650 nm for 10min.**
- **Discarding of the supernatant and use of the pellet for RAPIDEC® CARBA NP test as indicated by the manufacturer for use with bacterial colonies.**
- **Interpretation of the results.**

**CONCLUSIONS**

- The RAPIDEC® CARBA NP test can rapidly detect CP- K. pneumoniae directly from positive blood cultures.
- It requires standard laboratory equipment and minimum expertise.
- Its use may significantly contribute to infection control measures and appropriate antibacterial treatment.
- Optimization of the protocol is needed for the detection of CP-A. baumannii and P. aeruginosa directly from blood cultures.

**KEY POINTS**

- The RAPIDEC® CARBA NP test rapidly detects carbapenemase-producing K. pneumoniae directly from positive blood cultures.
- The easy-to-use test may significantly contribute to infection control measures and appropriate treatment.
Fast carbapenemase detection by RAPIDEC® CARBA NP

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INTRODUCTION
Emergence of carbapenemase-producing Gram-negative bacilli (CPGNB), including Enterobacteriaceae (EB), Pseudomonas (PSE) and Acinetobacter baumannii (AcB) is a major public health concern. Their early detection in colonized or infected patients allows to take appropriate infection control measures and possibly to adapt the antimicrobial therapy.

The RAPIDEC® CARBA NP test (RAPIDEC) has been industrialized by bioMérieux to answer this need. This test, based on the principle of the CARBA NP test (CNP) described by Nordmann et al. (Dec 2012, AAC, vol.56 6437-6440 ; Jul 2014, JCM, vol.52 2359-2366), relies on an acidification following the imipenem hydrolysis by CPGNB. The intended use of this test is the rapid detection of carbapenemase-producing strains, and specially carbapenemase-producing Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii. This study aimed to evaluate the preliminary performance of RAPIDEC® using a well characterized strain collection in comparison with the CNP test and the currently CLSI recommended modified Hodge test (MHT).

MATERIAL AND METHODS

Table 1. Distribution of the strains

<table>
<thead>
<tr>
<th>No of strains</th>
<th>Resistance type</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPGNB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>EB</td>
<td>KPC</td>
</tr>
<tr>
<td>16</td>
<td>NDM-I</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>RAPIDEC</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>RAPIDEC</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>NDM-I</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>OXA-48</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GE-5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NDM-1/OXA-48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>nontB</td>
<td>GE-5</td>
</tr>
<tr>
<td>2</td>
<td>RAPIDEC</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>nontB</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>nontB</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>nontB</td>
<td></td>
</tr>
<tr>
<td>Non CPGNB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>ampC</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>EB AMP</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>R due to porin</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>wild strains</td>
<td></td>
</tr>
</tbody>
</table>
| Sensitivity of detection of CPGNB
| Total of | Non | Total of | Non | Total of | Non |
| CPGNB | CPGNB | CPGNB | CPGNB | CPGNB | CPGNB |
| 116 | 113 | 100 | 100 | 100 | 96 |
| 3 | 2 | 2 | 2 | 2 | 3 |

RESULTS

Table 2: Number of strains detected positive or negative by the RAPIDEC®, CNP and MHT tests

| Table 3: Performance of the different tests |

Table 4: Number of strains positive with RAPIDEC® depending on the inoculum

Table 5: Number of strains detected positive or negative by the RAPIDEC®, CNP and MHT tests

Table 6: Sensitivity of detection of CPGNB

Table 7: Specificity of detection regarding CPGNB

Table 8: Specificity of detection concerning non-CPGNB

Table 9: Sensitivity of detection concerning non-CPGNB

DISCUSSION

RAPIDEC®, CNP and MHT allowed detection of 113 (97.4%), 100 (86.2%) and 86 (74.1%) of the 116 CPGNB, respectively. Regarding the detection of the non-fermenting (Non-CPGNB) rods and especially the PSE, only 2 PSE producing a GES carbapenemase were not detected, one by both RAPIDEC® and CNP and the second was not detected by the CNP test only.

CONCLUSION

This study showed that RAPIDEC® CARBA NP test allowed a rapid, standardized, sensitive and specific detection of the carbapenemase-producing Gram-negative bacilli, that could include Acinetobacter, contrary to the CARBA NP test. The preparation of the test and especially the adjustment of the inoculum is very important to obtain a good specificity. The modified Hodge test was much less sensitive and specific. RAPIDEC® should greatly contribute to the fight against carbapenemase thanks to its easy implementation by any lab, its low cost and its very good performance.
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