Selection of Publications

SCREENING AND CONFIRMATION OF CARBAPENEMASE PRODUCERS
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.

STRATEGY FOR SCREENING AND CONFIRMING CARBAPENEM RESISTANCE

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chromID™ CARBA
chromID™ CARBA SMART
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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
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

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.
Saegeman, 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.
Zarakolu, 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;73:35-40

Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae.
Girlich D, 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;50:5102-4
**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
Dévigne L, et al.
Evaluation of Multiple Methods for the Detection of Gastrointestinal Colonization of Carbapenem-Resistant Organisms from Rectal Swabs

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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 ertapenem for selection; a modified CDC broth enrichment method using ertapenem and vancomycin for selection; a direct MacConkey plate with ertapenem 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 ertapenem 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 carbapenems 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 the broth enrichment step.

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

Among the culture-based methods, chromID™ CARBA showed the best performance for screening of carbapenem-producing Enterobacteriaceae (CPE).
Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods


This state-of-the-art review covers the current status of surveillance methods for the detection of intestinal carriage of carbapenemase-producing organisms (CPOs).

The authors made a systematic review of all surveillance methods and give clear definitions and recommendations regarding the context of surveillance, the definition of carbapenemases and carbapenem resistance, as well as the classification of 8 culture media, confirmation reagents and molecular methods for CPO screening.

In particular, the authors highlight the importance of screening for intestinal carriage for the development of infection control strategies and the effective control of infections due to CPOs. Sites that have implemented a “bundle approach” to control the spread of CPOs, including screening of carriers, have been found successful in decreasing carriage rates.

Culture-based screening methods are readily available in clinical microbiology laboratories, and chromogenic media have made culture-based screening more convenient, despite long turnaround times. Based on their analysis in this review, the authors favor the use of chromID™ CARBA. However, if a hospital is located in a geographic area with a high incidence of OXA-48, the clinical microbiology laboratory should strongly consider the use of Supercarba or the addition of an OXA-48-specific medium such as chromID™ OXA-48 medium. A biplate, chromID™ CARBA SMART, containing chromID™ CARBA and chromID™ OXA-48 is also available.

Agar-based and colorimetric tests are generally affordable and able to detect the presence of “new and emerging” carbapenemases before they are characterized. The colorimetric Carba NP test is one of the recommended tests for confirmation of carbapenemase production in pure isolates by the CLSI and EUCAST, and can be performed in most microbiology laboratories, with no additional equipment. Agar-based procedures always require confirmatory testing to detect the type of bla gene present after a potentially resistant isolate is detected. Clinical microbiology laboratories may choose an agar-based screen with follow-up molecular testing, or a molecular method followed by culture if further investigation of the isolate is required.

This review sets out the strengths and limitations of available methods to help infection control practitioners and clinical microbiologists determine the most suitable approach for the infection control needs in their medical facility. It concludes that a proactive approach to halt the spread of carbapenemase producers is needed to prevent and control infections caused by CPOs and to protect public health.

“Screening for intestinal carriage of CPOs is of significant importance for the development of infection control strategies”
Faecal carriage of carbapenemase-producing Gram-negative bacilli in hospital settings in southern France

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(f) Laboratoire de Biologie Polyvalente, CH du Pays d’Aix, Aix-en-Provence, France.
(g) Laboratoire de Biologie Polyvalente, CH Saint-Jean, Perpignan, France.
(h) Laboratoire de Biologie Polyvalente, CH Edmond Garchin, Aubagne, France.
(i) Service des Maladies Infectieuses et Tropicales, CHU Carémeau, Nîmes, 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<sup>®</sup> easyMAG™ and NucliSENS EasyQ<sup>®</sup> 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<sup>3</sup> CFU) and a high inoculum (10<sup>6</sup> 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 <i>P. aeruginosa</i> (1.5%, n=17), <i>Enterobacteriaceae</i> (0.7%, n=8) and <i>A. baumannii</i> (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 specificity 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

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The study evaluated the hypothesis of a carbapenemase-producing Enterobacteriaceae (CPE) reservoir in a geriatric/chronic care population. 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 with 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 >0.5 µg/ml and/or temocillin MIC >165 µg/ml and each Eswab was analyzed by Check-Direct CPE for the detection of blaKPC, blaOXA-48, blaVIM/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 OXA-48, KPC and NDM should be considered.

“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.”

**KEY POINTS**

- chromID™ OXA-48 recovered the only OXA-48 producing *K. pneumoniae* strain.
- The recovery rate of CPE does not increase with the use of an enrichment broth or with one day more incubation of chromID™ media.
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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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™ CARBA, as well as the chromID™ CARBA chromogenic medium, was compared to the broth enrichment method (5 ml TSB plus 10 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 efficacy 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.

“When [chromID™ OXA-48 is] used with chromID™ CARBA, this combination of media potentially offers a highly effective solution for detection of Enterobacteriaceae with any commonly encountered carbapenemase.”

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.
* For a more cost-effective solution versus conventional media, 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

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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: Oxoid Brilliance ESBL, Oxoid Brilliance CRE, chromID CARBA, CHROMAgar Colorex C3Gr and CHROMAgar Colorex 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 KPC (Se 83.7% 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.

“Overall, […] chromID™ CARBA was the most sensitive and specific chromogenic media evaluated for the detection of CPGNB…”
Performance of chromID™ CARBA Medium for Carbapenemases-Producing Enterobacteriaceae Detection during Rectal Screening

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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”
This study compared the performance of chromID™ OXA-48 in combination with chromID™ CARBA on characterized strains and clinical samples in comparison with SUPERCARBA medium in order to determine the limit of detection for OXA-48 producing Enterobacteriaceae.

A total of 117 strains (characterized by PCR) were tested, including 57 OXA-48 and OXA-48 variant producers. The limit of detection (LOD) was determined by testing five dilutions ranging from $1 \times 10^2$ to $1 \times 10^6$. Then, $100 \mu l$ of each inoculum was plated on the three screening media. Viable bacteria were counted after 24 hours incubation at 37°C.

In the second part of the study, 120 clinical rectal swabs and 30 stool samples containing no OXA-48 like producers were inoculated for the determination of the specificity of the screening media for detection of OXA-48. A quantity of $100 \mu l$ of suspension were plated on each screening medium.

The determination of the LOD shows chromID™ OXA-48 has a high sensitivity ranging from 91.2% to 96.5% respectively for a low and a high inoculum. Regarding the sensitivity and specificity for detection of OXA-48 producers, SUPERCARBA has the highest sensitivity, ranging from 93–100%, respectively (for a low and a high inoculum) and 52.5% specificity. chromID™ OXA-48 also has a high sensitivity (see above), and the highest specificity with 100%. chromID™ CARBA has a low sensitivity (<30%) for both low and high inocula and 67.5% specificity.

In conclusion, chromID™ CARBA shows a weak sensitivity for detection of OXA-48 producers but is a powerful tool for detection of all other class of CPE. chromID™ OXA-48 is a sensitive medium for detection of OXA-48 producers. The combination of chromID™ CARBA/chromID OXA-48 offers the highest sensitivity for detecting any type of carbapenemases.

“The potential use of the novel chromID™ OXA-48 medium shall be for controlling an ongoing outbreak of OXA-48 producers or in combination with the chromID™ CARBA.”
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.”
A comparison of four chromogenic culture media for carbapenemase-producing *Enterobacteriaceae*

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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*”. 
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 Magalong, E Villena, DA Boyd, L. Mataseje, P Lo, M. Mulvey, T Mazzulli, SM Poutanen
Mount Sinai Hospital/UHN, University of Toronto, Toronto; William Osler Health Sciences Centre, Brampton; National Microbiology Laboratory, Winnipeg, Canada

ABSTRACT
Objectives: CPO screening agars are typically poorly specific and can lack sensitivity for various CPO. The CARBA-SMART bi-plate places OXA agar (designed to be sensitive and specific for class D CPO) side-by-side CPO agar (sensitive for classes A+B but not class D CPO) to improve overall chromID sensitivity. Similar to chromID CPO agar, the RAPIDEC CARBA-NP is sensitive for class A+B but not class D CPO. This study compared CPO detection from surveillance swabs by the Oxoid ESBL/meropenem disc screen algorithm to the combined abilities of RAPIDEC CARBA-NP agar 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 (30 µL) to 3 agars: Oxoid ESBL and 2 lots of CARBA-SMART, with 4 lots used over the study period. Oxide-neg isolates from ESBL were tested by meropenem disc using the cutoff of <25mm (sensitivity 100%, specificity 85%), while from CARBA-SMART, burgundy E. coli (EC) or teal (usually K. pneumoniae (KP) or E. cloacae (ECL) etc.) colonies were tested directly by RAPIDEC (1/species/surveillance swab - duplicates referred). By 2h, distinct changes in RAPIDEC from red to orange or yellow were taken as CPO-positive, with RAPIDEC negatives confirmed by PCR using either Cepheid Xpert CARBA-R or conventional PCR at NML.

Results: A total of 33 (5.6%) from 928 swabs grew CPO from both ESBL and CARBA-SMART agars. Results from 1836 plated to CPO/ESBL to OXA agar, respectively were: no growth [1500 (81.7%)]; 1688 (92.1%); no significant growth [246 (13.4%)/149 (8.1%); 18/19 E. faecium (EF; dark blue), 40/76 S. haemolyticus, 166/49 P. aeruginosa, 22/5 S. maltophilia (no colour)]; potentially significant (PS) growth from 40 (4.3%) swabs [170 (9.2%)]; 25/10 EC, 5/0 ECL]. Of the 170/24 PS, 45/9 unique isolates (total 54) were tested by RAPIDEC. From CPO, 34/45 were RAPIDEC-positive (14/15 EC/NDM, 9/9 KP/NDM, 5/5 ECL/NMC, 2/2 KP/VIM, 1/1 each ECE/NDM/VIM, KP/NDM/VIM, KP/NDM/VIM/OXA48, and ECL/NDM+VIM+OXA48), 5/45 falsely RAPIDEC-negative (TN; 3/3 KP/OXA48, 1/1 EC/OXA48, 1/15 EC/NDM) and 6/45 were RAPIDEC-true negatives (TN; 4 KP, 1 EC, 1 teal EF). As anticipated from prior studies, 8/9 CPO from OXA were FN by RAPIDEC (5 KP-OXA48, 2 EC-OXA48, 1 KP-OXA48/NDM/VIM), while 1 EC was RAPIDEC-TN.

Conclusions: While CARBA-SMART appeared equivalent to ESBL in detecting CPO from surveillance swabs, laboratories considering implementing this otherwise useful combination need to take into account the RAPIDEC CARBA-NP limitation of its inability to detect all OXA48-like CPO and occasionally blaNDM carried in E. coli.

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 detects all possible genotypes. CPO evolve rapidly with novel types appearing without warning, while other types diversifying via genetic drift at rates that have, in some instances, unknowingly reduced PCR detection sensitivities. Routine PCR detection from surveillance swabs is cost-prohibitive, especially in low prevalence settings. But PCR is an important tool for large laboratories to enable rapid CPO detection (<4h) direct from swabs in high-risk situations, albeit if only for the limited array of more common CPO genotypes. For routine CPO surveillance, swabs first undergo selective culture. Past studies have found most “CPO-specific” screen agars to be poorly specific and to lack sensitivity for various CPO types. To increase the performance of the chromID CPO agar, bioMérieux created a bi-plate, the chromID CARBA-SMART, using “OXA” agar side-by-side their original CPO agar to improve overall CPO detection capabilities. OXA agar is claimed to specifically improve detection of class D CPO for which CPO agar had proven insensitive. Similar to chromID CPO agar, bioMérieux’s RAPIDEC CARBA-NP, a rapid (<4h) phenotypic CPO detection test, was shown to be sensitive for A and B but not class D CPO. This study compared the combined use of these two products for CPO detection from surveillance swabs. Outcomes were compared to those obtained using the Oxoid ESBL/meropenem disc screen algorithm when RAPIDEC CARBA-NP was used directly from colonies on the CARBA-SMART agar, with the proviso that RAPIDEC-negatives, especially from OXA agar, would require alternative testing such as PCR to rule out CPO.

Figure 1a: Examples of bioMérieux’s CARBA-SMART agar (A) mixed growth of blaVIM Citrobacter freundii (dark blue), blaNDM Klebsiella pneumoniae (blue/ turquoise) and blaVIM E. coli (burgundy pink) on CPO agar inhibited on OXA agar, (B) pure growth of blaNDM E. coli on CARBA agar inhibited on OXA agar, (C) pure growth of blaVIM E. coli on OXA agar but inhibited on CARBA agar, (D) an example of breakthrough, in this case, a pure growth of non-CPO Acinetobacter baumannii.

Figure 1b: The RAPIDEC CARBA-NP a rapid (<3h) phenotypic test for detecting carbapenemases in isolates, (left) a red or no colour-change in well “e” indicates a positive test, (right) a red or no colour-change in well “a” indicates a negative test.
METHODS
Consecutive eSwabs (typically rectal +/- nasal specimens) received by the MSH/UHN laboratory for CPO surveillance were enrolled into the prospective CARBA-SMART study. To increase the CPO positivity in the study, a further 56 rectal swabs, previously processed for CPO at a collaborating laboratory in a high prevalence area that contained CPO or carbapenem-resistant non-CPO were included in the study.
Referred-in swabs were inoculated into fresh Copan eSwabs tubes at the time of planting so that all swabs could be simultaneously and equally inoculated in parallel onto each side of 2 distinct lots of the bioMérieux’s CARBA-SMART bi-plate under study and to the Oxoid ESBL agar used in the routine laboratory algorithm. Over the study period, 4 distinct lots of CARBA-SMART were evaluated (~500 plates each lot). The parallel plating was performed automatically by the WASP system which deposited and streaked-out 30µl of specimen in Amies liquid medium onto each agar type. All inoculated/streaked CPO screen plates were placed at 37°C simultaneously for overnight incubation.

1) Routine laboratory algorithm: Oxidase-negative isolates grown on Oxoid ESBL agar were tested by meropenem disc diffusion (10µg meropenem disc on Oxoid’s Mueller-Hinton Plus agar interpreted using previously validated breakpoint of ≤25mm to indicate a possible CPO; applicable only to Enterobacteriaceae). Any screen-positive isolate was subjected to identification to the species level using VITEK MS Plus MALDI-TOF, to ROSCO KPC+MBL+OXA48 Confirm kit and/or Cepheid Xpert CARBA-R PCR and/or conventional PCR at NML for detection of carbapenemases and sequencing, if needed, to determine precise alleles in rarer CPO types.

2) bioMérieux chromID CARBA-SMART agar/RAPIDEC CARBA NP study algorithm: Any oxidase-negative Gram-negative bacillus (Enterobacteriaceae - burgerundy pink (Escherichia coli), teal (Klebsiella, Enterobacter- or Citrobacter-like species, etc.) or brown (Proteus mirabilis, Morganella morganii, Providencia spp.,) growing on either side of the CARBA-SMART bi-plate was tested directly from the agar by RAPIDEC for phenotypic evidence of carbapenemase-production. The RAPIDEC was interpreted as positive if by 2h, distinct changes in test well colour compared to control well colour from red (negative) to orange or yellow had taken place. Any suspect isolate was also subjected to the full routine laboratory algorithm. Other colony types growing on the CARBA-SMART bi-plate were documented but only minimally identified.

CARBA-SMART and RAPIDEC CARBA-NP results were unblinded for correlation with final laboratory results only after completion of confirmatory CPO testing. Sensitivities and specificities with 95% confidence intervals were calculated using www.graphpad.com

RESULTS
A total of 928 surveillance swabs were planted in parallel by WASP to ESBL and 2 lots of CARBA-SMART agar bi-plates (lots 1 and 2) were used were used during 1st half of study; lots 3 and 4 during 2nd half of study). Of these, 33 (3.56%) swabs grew >1 confirmed CPO from the ESBL agar and either CARB or OXA agars on the CARBA-SMART bi-plate (Table 1).

Results from the 1846 CARB and the 1847 OXA agars plated, respectively, were:

• No growth was obtained on 1500 (81.3%) CARB agars and 1688 (91.4%) OXA agars
• Non-specific breakthrough growth of Gram-positive species, oxidase-positive Gram-negative species, or intrinsically carbapenem-resistant Gram-negative species was detected on 246 (13.4%) CARB agars and on 149 (8.1%) OXA agars, and included:

• CARB: 18 E. faecium (dark blue), 40 S. haemolyticus (white opaque), 166 P. aeruginosa (cream, green, or brownish, +/-indescence/ diffusible pigment in underlying agar), 22 S. maltophilia (colourless)
• OXA: 19 E. faecium, 76 S. haemolyticus, 49 P. aeruginosa, 5 S. maltophilia (isolates exhibited same morphologies on OXA as on CARB agar)

From all CARBA-SMART with potentially significant growth, 45 unique isolates from CARB and 9 from OXA were tested by gold standard PCR algorithms and by RAPIDEC CARBA-NP (data from duplicates tested was excluded for this presentation). Of these, 47 unique CPO were isolated based on PCR. RAPIDEC was positive in 31/47 unique CPO, and negative in 16 CPO and 7 non-CPO (below) for an overall phenotypic CPO detection sensitivity of 66% (95% CI: 51.6-77.9). Although only small numbers of non-CPO were tested by RAPIDEC, specificity was 100% (95% CI: 60-100).

Of the 45 isolates from CARB, PCR confirmed 39 CPO and 6 non-CPO, while RAPIDEC was positive in 31/39 (79.5%) CPO and 0/6 non-CPO

<table>
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<th>Outcomes from CPO surveillance swabs plated prospectively to 4 production lots of bioMérieux’s CARBA-SMART agar</th>
<th>No. swabs</th>
<th>No. isolates over 4 lots</th>
<th>CARBA-SMART chromID CARBA / OXA agar results from lots by lot</th>
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<td>Breakthrough non-CPO isolates****</td>
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<tr>
<td>Confirmed CPO*****</td>
<td>33</td>
<td>166</td>
<td>37/6</td>
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</table>

* Occasionally, surveillance swabs had insufficient fluid for plating by WASP to both CARB and OXA agars of the CARBA-SMART bi-plate
** Non-significant/non-specific species grown on CARB and OXA agars included S. haemolyticus, E. faecium, S. maltophilia, P. aeruginosa
*** Potentially significant isolates included only Enterobacteriaceae isolates on either CARB or OXA agars
**** Breakthrough was any Enterobacteriaceae grown on either CARBA-SMART agar that was negative by confirmatory tests for CPO genes
***** Confirmed CPO includes overlapping duplicates from parallel testing of same swabs; some contained multiple CPO in different species
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

KEY POINTS
- chromID™ CARBA SMART is compatible with WASP® (COPAN).
- chromID™ CARBA SMART enables the easy detection and differentiation of CPO in 18-24h.
- Colonies on chromID™ CARBA SMART are large enough to allow confirmation with RAPIDEC® CARBA NP.

DISCUSSION & CONCLUSION

Use of bioMérieux’s CARBA-SMART as a CPO detection agar
- This prospective evaluation used consecutive surveillance swabs enriched with CPO-positive swabs from a high-prevalence institution and found CARBA-SMART agars’ combined CPO detection capabilities equivalent to the comparator ESBL agar algorithm.
- CARBA-SMART was WASP compatible thus simple to use, and chromogens enabled easy differentiation of mixed CPO populations, and in some cases, separated out distinct genotypes according to their ability to grow or be inhibited on the 2 agar formulations.
- OXA agar improved the overall chromID CPO detection sensitivity as compared to CARB agar alone, since blaOXA48 CPO that failed to grow on CARB were easily detected on OXA agar.
- To identify CPO on CARBA-SMART, as with ESBL agar, oxidase testing was used to eliminate oxidase-positive Gram-negative species and MALDI-TOF was used to rule out species with intrinsic resistance, after which all remaining Enterobacteriaceae were tested for CPO.
- All CPO grew by 18-24h and there was negligible breakthrough of non-CPO Enterobacteriaceae on OXA agar; however CARB agar often grew non-CPO Enterobacteriaceae [28/194 potential CPO were non-CPO E. coli or K. pneumoniae; positive predictive value of growth for CPO = 85.6% (95% CI: 79.9-89.9)].

Combined use of bioMérieux’s CARBA-SMART agar and bioMérieux’s RAPIDEC CARBA-NP
- Colonies were sufficiently large on CARBA-SMART to allow for the heavy inoculum required to enable set up of the useful phenotypic RAPIDEC test directly from the primary agars.
- While RAPIDEC detected all class A in the study (limitation: no prospective blaKPC CPO were encountered during the study period), it missed 1 blaNDM in E. coli and all 12 OXA48 CPO studied. Therefore, alternative CPO detection tests are mandatory for Enterobacteriaceae that test RAPIDEC-negative if any class D CPO and all class B CPO are to be identified.
Retrospective Evaluation of the Performance of the chromID CARBA-SMART Bi-Plate to Detect Carbapenemase-Producing Organisms (CPO)

Mount Sinai Hospital/UHN, University of Toronto, Michener Institute, Toronto; William Osler 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 as high as possible to minimize confirmatory rule-out work. This retrospective study evaluated performance of bioMérieux’s chromID CARBA-SMART, a chromogenic bi-plate where CARB 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, 3 NMC/IMI, 2 GES; 80 class B: 73 NDmA, 6 VIM, 1 IMP; 26 class D; OXA48, OXA181, OXA232, OXA244, 7 class B+D; NDMA+OXA181, NDM+OXA232) and 38 non-CPO with mixed mechanisms (derepressed-ampC, ESBL, ompC/ompF or ompK35/ompK36 mutants, 1 cphA1, OXA252). Standard saline 0.5-MacFarland equivalent suspensions, prepared using colonies growing closest to ertapenem discs placed on MacConkey agar sub-cultures for selective pressure, were transferred to emptied Copan eSwab tubes for automated inoculation (10μL/side) to CARBA-SMART by the WASP 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 CARB agar, 2) class D CPO on OXA agar, and 3) overall CPO detection of both agars combined. 95% confidence intervals (CI) were calculated using www.graphpad.com.

Results: Only 1 NDM+ P. mirabilis and 1 OXA48+ E. coli were not detected by either agar on CARBA-SMART resulting in an overall Sn (95% CI) of 99.1% (96.6-99.97). The CARB agar grew all but 6/221 CPO (1 NDM+ Proteus mirabilis and 5 OXA48-type Escherichia coli (2 OXA181, 3 OXA48)) resulting in a CPO detection Sn (95% CI) was 97.3% (94.1-98.9), and Sn by class was: A (100%; 95.9-100), B (98.8%; 92-99.99), and D including B+D (84.9%; 68.6-93.8). The OXA agar grew all but 1/53 class D (1 OXA48 E. coli that also failed on CARB) and also grew 1 class A (SME+ Serratia marcescens) and 2 class B (1 NDM+ Acinetobacter baumannii, 1 VIM- Pseudomonas putida) CPO; corresponding Sn (95% CI) was 97% (83.4->99.99) 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 CARB and 1 on OXA agars, resulting in Sp (95%CI) of 68.4% (52.5-81) and 98.2% (95.4-99.5), respectively.

Conclusions: This evaluation of the CARBA-SMART chromogenic bi-plate found the OXA agar to complement the chromID CARB agar as 4/5 OXA48-type CPO that were not detected on CARB 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 detects all possible genotypes. CPO evolve rapidly with novel types appearing without warning, other 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 though PCR is an important tool for large laboratories to enable rapid CPO detection (<4h) 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 agars to be poorly specific and to lack sensitivity for certain CPO genotypes. Thus, for maximum sensitivity (at 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 cepodoxime), 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 CARB agar was insensitive to OXA48-like CPO), bioMérieux created a bi-plate, the chromID CARBA-SMART (Figure 1). This bi-plate consists of “OXA” agar side-by-side the original chromID CARB 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.
METHODS

Table 1 (below) describes the 259 species-diverse clinical isolates selected for the study. Prior to the study, all isolates were fully-characterized for genetic content by conventional PCR for ESBL and CPO genotypes with sequencing, as needed, to elucidate allele variations or contributory mechanisms such as mutations in ompC/ompF, ompK35/ompK36 or gene promoters etc. Each isolate was identified to the species-level by MALDI-TOF (bioMérieux’s VITEK MS Plus system). Phenotypic expression of carbapenemases had been measured by standard meropenem disc diffusion using a screen breakpoint of <25mm. Reactions to boronic and dipicolinic acid inhibitors had been determined alongside temocillin susceptibilities (ROSCO’s KPC+MBL+OXA48 confirm kit). Most isolates were derived from distinct patients, however, there were cases where multiple genotypes were present in distinct isolates of different genera/species within the same patient. No duplicate isolates with the same identity and gene content were used from any one patient specimen.

On recovery from -80°C, all isolates were blinded to prevent bias. Furthermore, on initial isolation and on second subculture prior to testing on bioMérieux’s CARBA-SMART agar, all isolates regardless of CPO/non-CPO status, were plated to MacConkey CV agar with an ertapenem disc placed in the main inoculum to maintain selective pressure.

At the time of CARBA-SMART testing, standardized saline 0.5-MacFarland equivalent suspensions were prepared using colonies growing closest to the ertapenem discs on the MacConkey agar. The suspensions were then transferred to emptied Copan eSwab trans-tubes for automated inoculation (10μL/side) to CARBA-SMART by the WASP system. After incubation at 37°C overnight (~4pm-10am), the quantity, colour and size of colonies were documented independently by 5 readers. Individual reads were later correlated to identify error. PCR was used to confirm genotypes in discrepant cases. If needed, repeat testing with isolates freshly recovered from the frozen stock was performed.

Final consensus data was analyzed for sensitivity and specificity for detection of all CPO on CARB agar, for detection of class D CPO on OXA agar, and for overall CPO detected on both agars combined. 95% confidence intervals (CI) were calculated using www.graphpad.com.

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<th>Ambler (No.)</th>
<th>CPO Genotypes (No.)</th>
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<td></td>
<td></td>
<td>blaOX4232 (4)</td>
<td>blaOX4232 (4)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Klebsiella pneumonia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blaOX4244 (1)</td>
<td>Escherichia coli</td>
<td>1</td>
</tr>
<tr>
<td>Non-CPO (38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ompC-ompF (4)</td>
<td>Entenobacter cloaceae</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ompK35-ompK36 (6)</td>
<td>Klebsiella pneumonia</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Weak OXY promoter (1)</td>
<td>Klebsiella oxytoca</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Other mechanisms (26)</td>
<td>Entenobacteriaceae</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>blaOX4232 (1)</td>
<td>Shewanella putrifaciens</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Retrospective Evaluation of the Performance of the chromID CARBA-SMART Bi-Plate to Detect Carbapenemase-Producing Organisms (CPO)

RESULTS

CARBA-SMART performed well in this study (Table 2). Together the agars detected most of the wide range of genetically diverse challenge CPO, as evidenced by an overall sensitivity (95% CI) of 99.1% (96.6-99.97) (Table 3). Only 1 Proteus mirabilis (blaNDM) and 1 Escherichia coli (blaOX48) were repeatedly missed by both agars.

CARB agar grew all but 1/221 CPO (1 NDM+ F. mirabilis) and 5 OXA48-type+ E. coli (2 OXA181+ and 3 OXA48+) resulting in a CPO detection sensitivity (95%CI) of 97.3% (94.1-98.9). Sensitivities by class were: A (100%; 95.9-100), B (98.8%; 92->99.9), and D including B+D (84.9%; 66.9-93.8).

OXA agar grew all but 1/3 class D CPO (1 OXA48+E. coli) that also failed on CARBA. It also grew 3 non-D CPO including 1 class A (temocillin-resistant SME+ Serratia marcescens) and 2 class B (1 NDM+ Acinetobacter baumannii, 1 VIM+ Pseudomonas putida; possibly due to intrinsic mechanisms resistant to the proprietary antibiotic selective agent in OXA agar). Corresponding sensitivity (95% CI) for OXA was 97% (83.4->99.99) for class D only, and as expected, it was low for any CPO [15.8%; 11.6-21.3].

Of 38 non-CPO, 12 grew well on CARB (all Enterobacteriaceae) and 1 E. coli on OXA, resulting in specificities (95% CI) of 68.4% (52.5-81) and 98.2% (95.4-99.5), respectively (Tables 2 and 3).

Table 2. Summary of bioMérieux’s chromID CARBA-SMART bi-plate overall performance showing any detection of any CPO on CARBA agar and detection of OXA48-like CPO on OXA agar

CPO genotypes* Class No. (%)

<table>
<thead>
<tr>
<th>Class</th>
<th>No. tested</th>
<th>No. isolated on CARBA (any CPO)/ No. CPO expected to grow (%)</th>
<th>No. isolated on OXA/No. class D CPO expected to grow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 blaKPCs</td>
<td>A 99 (38.2)</td>
<td>99/99 (100)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>2 blaSME</td>
<td>A 4 (1.5)</td>
<td>4 (1.5)</td>
<td>1/0 (25)*</td>
</tr>
<tr>
<td>3 blaKPC-4-M1</td>
<td>A 3 (1.2)</td>
<td>3/3 (100)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>4 blaGES5</td>
<td>A 2 (0.8)</td>
<td>2/2 (100)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>5 blaNDM</td>
<td>B 75 (28.2)</td>
<td>72/75 (95.9)</td>
<td>3/5 (60)***</td>
</tr>
<tr>
<td>6 blaVIM</td>
<td>B 6 (2.3)</td>
<td>6/6 (100)</td>
<td>1/0 (16.7)***</td>
</tr>
<tr>
<td>7 blaIMP</td>
<td>B 1 (0.4)</td>
<td>1/1 (100)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>8 blaOX48-like</td>
<td>D+B 26 (10)</td>
<td>25/26 (96.2)****</td>
<td>1/0 (100) **</td>
</tr>
<tr>
<td>9 blaOX48-like +blaNDM</td>
<td>D+B 7 (2.7)</td>
<td>7/7 (100)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>Total CPO tested</td>
<td>221 (85.3)</td>
<td>218/221 (98.6)</td>
<td>32/33 (97) class D; 5/33 (1.6) non-D</td>
</tr>
<tr>
<td>Non-CPO</td>
<td>0 37 (14.3)</td>
<td>11/0 (28.7)******</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>Non-CPO (intrinsic cpaA)</td>
<td>0 1</td>
<td>1/0 (100)*****</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>Total Non-CPO tested</td>
<td>38 38 (14.7)/1 (2.6)</td>
<td>18 (23.7)/0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total isolates tested</td>
<td>259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* OXA growth reproducible in 1 blaSME. 5. marcescens atypically temocillin-resistant (WGS found no OmpCF/ampC mutations or B lactamases besides blaIMP)

** Reproducibly no growth from 0.5 McF suspension in 1 E. coli; only able to grow poorly after heavy colony inoculation

*** Reproducibly no growth from 0.5 McF suspension in 1. baumannii (blaNDM, CWA11) and 1 P. putida (blaNDM)

**** Reproducibly no growth from 0.5 McF suspension in 1 S. marcescens, 2 blaOXY promoter mutants; 6 mixed ESBL/ampC

****** Growth in all 11 non-CPO that grew on CARB was heavy; mechanisms: 2 ompCF, 2 ompK35/36, 1 blaOXY promoter mutants; 6 mixed ESBL/ampC

KEY POINTS

• chromID™ CARBA SMART is simple to introduce into routine practice for CPO growth in 18 hours.

Table 3. chromID CARBA-SMART performance by Ambler class

<table>
<thead>
<tr>
<th>Class</th>
<th>chromID CARBA agar</th>
<th>chromID OXA agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A CPO</td>
<td>108/108 (100%; 95.9-100)</td>
<td>1/108 (0.9%; &lt;0.01-5.5)</td>
</tr>
<tr>
<td>B CPO</td>
<td>79/80 (98.8%; 92.6-&gt;99.99)</td>
<td>2/80 (2.5%; 0.2-9.2)</td>
</tr>
<tr>
<td>D CPO*</td>
<td>28/33 (84.9%; 68.6-93.8)</td>
<td>32/33 (97%; 83.4-&gt;99.99)</td>
</tr>
<tr>
<td>ANY CPO</td>
<td>215/221 (97.3%; 94.1-98.9)</td>
<td>35/221 (15.8%; 11.6-21.3)</td>
</tr>
</tbody>
</table>

** When results from both agars are taken together, the CARBA-SMART missed only a single blaNDM Proteus mirabilis and a single blaOX48-like Escherichia coli

CONCLUSION & DISCUSSION

Use of bioMérieux’s CARBA-SMART as a CPO detection agar

• This retrospective CARBA-SMART evaluation, that used highly-diverse and well-characterized CPO and non-CPO, found the combined use of CARB and OXA agars to detect 99.1% of CPO overall while maintaining high specificities on OXA (98.2%) but not on CARB (68.4%) agars.

• Since 4 of 5 blaOX48-like CPO that failed to grow on CARB were easily detected on OXA agar, the CPO detection sensitivity of CARBA-SMART improved overall as compared to chromID CARB agar alone [97.3% (94.1-98.9) 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, reproducible growth on CARB agar of breakthrough non-CPO and growth on both OXA and CARB agars of oxidase-positive species, indicates that testing to eliminate oxidase-positive Gram-negative species and MALDI-TOF to rule out species with intrinsic resistance should precede or accompany testing of remaining Enterobacteriaceae for CPO.

• As CARBA-SMART is WASP compatible, and most CPO grew <18h, and since its chromogencs for differentiation of key general species are in keeping with common chromogenic agars used for urine culture, it would be simple to introduce into routine laboratory workflows.
 мероприятии

Цель

Карбапенем-резистентные Enterobacteriaceae (CRE) многолетние антибиотикорезистентные бактерии, которые могут быть связаны с медико-санитарным инфекционным процессом. Метаболический контроль и управление носителем CRE в многокамерных помещениях является необходимым для предотвращения и контроля возникновения инфекций. Дизайн этого исследования состоит в оценке эффективности этого средства EARID CARBA SMART для обнаружения CRE.

Методы

Этот исследовательский проект проведён в здании Университетской больницы на Канарских островах, в 667-местной больнице в Тенерифе, Испания, в период с 26 мая по 31 июля 2014 года.

Широко использовались грибковые и фарингеальные тесты, взятые от каждого пациента, который был направлен в интенсивную терапию (ИТ). Также использовались грибковые и фарингеальные тесты, взятые от каждого пациента, который был направлен в медицинские или хирургические отделения, где был ранее обнаружен CRE в клинических образцах. Образцы были выращены на EARID CARBA SMART селективно-цветном среде (био Мерчюэ), содержащейся в биоплите. Пластины были инкубированы при 37°C, а рост был отмечён через 24 и 48 часов.

В случае роста, идентификация и испытание антимикробной чувствительности были выполнены с помощью технологии VITEK™ (био Мерчюэ) и также дополнительного теста (модифицированный тест Ходж и фенотипический тест для обнаружения карбапенемазы).

Результаты

Мы подсчитали 562 образца от 348 пациентов. 163 из этих образцов были взяты от пациентов, направленных в ИТ. Мы обнаружили 22 образца с CRE, 21 из которых вырастили в грибковых тестах и 1 в фарингеальных тестах.

Общая распространенность: 3,91%. Распространенность по отделениям: General surgery 7,55% (4), Neurosurgery 0,23% (2), Internal Medicine 5,78% (1), Oncology-Hematology 0,55% (2), ICU 7,55% (4).

В каждом из тех случаев, когда EARID CARBA SMART был положительным, это было подтверждено как CRE (100% специфичность). Относительно пациентов, направленных в ИТ, Макконкик мог не вырасти CRE в любом из отрицательных случаев EARID CARBA SMART (100% специфичность) и все положительные случаи CRE вырастили на EARID CARBA SMART и в Макконкик агаре (био Мерчюэ).

Все CRE, которые были обнаружены, были классифицированы как OXA-48-тип карбапенемазной Enterobacteriaceae.

Заключение

EARID CARBA SMART селективно-цветное средство (био Мерчюэ) позволяет быструю идентификацию CRE с 100% специфичностью и эффективностью и он полезен для ранней идентификации носителей, сбережения микробиологических затрат.

Ключевые моменты

- EARID CARBA SMART обнаруживает все OXA-48 производители с 100% специфичностью и 100% специфичностью в этом исследовании.
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 (CPE) are emerging as a growing challenge in health care facilities (1). 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 (2).

Carbapenemase-producing pathogens have been associated with high rates of morbidity and mortality particularly among critically ill patients with prolonged hospitalization. It is also of note that the carbapenemase genes harbored by CPE are mostly transposon- and/or integron-encoded determinants that can easily disseminate to other enterobacterial strains and species. These facts suggest the need to implement adequate preventive measures, including active surveillance, in order to detect infected patients and carriers with multidrug-resistant isolates and contain the spread of these pathogens. (3)

The direct detection of CPE carriers, by selective chromogenic medium, is a useful tool for rapid and inexpensive screening of patients for CPE and is also available for daily use in many laboratories. Culture techniques for screening CPE have been tested, including methods that use in-house-prepared selective media, such as TSBs 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 translucid colonies with a pink to burgundy center, while Klebsiella, Enterobacter, Serratia, Citrobacter (KESC group) species produce bluish-green 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: i) detection and differentiation of a previously well-characterized collection of CPE with various enzymatic resistance mechanisms and ii) screening for CPE carriers.

METHODS

Overall 60 CPE (47 KPC, 11 NDM, 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, 8 TEM-type, 6 CMY-16, 2 porin loss plus ESBL or AmpC hyper-producing isolates) were plated onto chromID™ CARBA medium. Two different inoculum sizes (10⁵ CFU/µl and 10⁶ 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 48h.

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 in air 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 meropenem disc and 5 ml of TSB containing a 10 μg meropenem disc. The broths were 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 37°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 ratios of CPE/non-CPE (1:100; 1:1,000; 1:10,000) in saline, 1 μl of the suspension was inoculated onto chromID™ CARBA agar. All plates were incubated in air at 37°C and the average number of colonies was recorded after 24h.

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).

Figure 1.

Figure 2.

1. E. coli (KPC+)
2. E. coli (KPC+)
3. E. coli (KPC+)
4. E. coli (KPC+)
5. K. pneumoniae (KPC+)
6. E. cloacae (VIM+)
7. E. cloacae (VIM+)
8. E. coli (CTX-M+)
9. E. coli (ESBL+)
10. K. pneumoniae (KPC+)
Evaluation of chromID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae

Figure 3. Mixed cultures in different ratios of CPE/non-CPE (1:100; 1:1,000; 1:10,000)

E. coli (KPC+)/ K. pneumoniae (CTX-M+)

K. pneumoniae (KPC+)/ E. coli (CTX-M+)

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 10^2 CFU/spot for high and low inoculum, respectively.

Out of the 400 rectal swabs analysed, 32 were positive with both routine 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 Aeromonas spp., 10 Pseudomonas spp., 1 Stenotrophomonas maltophilia and 1 Enterococcus faecium).

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

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 carbapenems MIC, 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 lower concentrations.

REFERENCES
(5) Centers for Disease Control and Prevention. 2009. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, Klebsiella spp. and E. coli from rectal swabs. Centers for Disease Control and Prevention, Atlanta, GA.

KEY POINTS
- chromID™ CARBA allows isolation of CPE strains from mixed cultures.
- chromID™ CARBA shows better recovery than routine methods in cases of low CPE bacterial load.
First evaluation of chromID® OXA-48 agar - a new chromogenic medium for detection of Enterobacteriaceae-producing OXA-48 carbapenemase

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INTRODUCTION
The spreading of strains producing OXA-48 carbapenemase is an emerging public health problem, particularly in Europe and throughout the Mediterranean. These strains are responsible for outbreaks and are often difficult to detect because of their low level of resistance expression. The purpose of this study is to evaluate the performance of chromID® OXA-48, a new chromogenic medium developed for the specific detection of Enterobacteriaceae (EB) producing OXA-48 carbapenemase (OXACPE). To assess the sensitivity and specificity, this medium was compared with internal collection strains to other available chromogenic media dedicated to the detection of carbapenemase producing strains (CPE): chromID® CARBA (bioMérieux), Brilliance CRE (Oxoid) and Colorex™ KPC (Diagnostics BioMed). Specificity was also assessed with clinical specimens plated on the chromID™ OXA-48 and chromID™ CARBA. Status of clinical samples was defined by direct PCR testing.

METHOD

Principle
The chromID® OXA-48 medium allows selective detection of the OXACPE by a coloration of the colonies thanks to the use of chromogenic substrates.

Reading and interpretation
• positive OXACPE: growth with characteristic coloration of the colonies (see figure 2)
• negative: no growth, growth of colourless colonies or colonies without characteristic coloration. chromID® CPS was used as growth control for internal collection strains.

Clinical sample
• Origin: Hospital centres from Aix-en-Provence, Alès, Aubagne, Montpellier, Nîmes, Perpignan and Toulouse (France)
• 1135 Specimens: 774 stools and 361 rectal swabs were collected from February 1 to April 30, 2012.

Table 1. Distribution of the internal collection strains

<table>
<thead>
<tr>
<th>Category</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB producing OXA-48 type carbapenemase</td>
<td>33</td>
</tr>
<tr>
<td>non OXACPE</td>
<td>1031</td>
</tr>
<tr>
<td>EB producing OXA-181 type carbapenemase (OXA-48 variant)</td>
<td>4</td>
</tr>
<tr>
<td>non OXACPE</td>
<td>1027</td>
</tr>
<tr>
<td>EB producing other carbapenemase</td>
<td>35</td>
</tr>
<tr>
<td>non OXACPE</td>
<td>1090</td>
</tr>
<tr>
<td>EB producing ESBL or gram negative bacteria over producing cephalosporinase (HLCase)</td>
<td>36</td>
</tr>
<tr>
<td>non OXACPE</td>
<td>1091</td>
</tr>
<tr>
<td>EB carbapenem resistant by impermeability</td>
<td>14</td>
</tr>
<tr>
<td>non OXACPE</td>
<td>1077</td>
</tr>
<tr>
<td>Wild type strains:</td>
<td>56</td>
</tr>
<tr>
<td>non fermentative Gram negative OXA-23</td>
<td>10</td>
</tr>
<tr>
<td>Vancomycin Resistant Enterococci, Van A or Van B</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 2. Mixed culture of OXACPE: K. pneumoniae (green colonies), E. coli (pink colonies).
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 \( \times \) 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 Colorex KPC are due to a lack of selectivity of the 2 media regarding EB strains with porin loss or producing HL.

Clinical samples: These 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 OXACPE. 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 OXACPE, 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.

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).
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 β-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-β-lactamase (NDM); Verona integron-encoded metallo-β-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
ARTICLES

A two-centre evaluation of RAPIDEC® CARBA NP for carbapenemase detection in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter spp.*
Kabir MH, et al.
*JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY* 2016;71:1213-6

Evaluation of the RAPIDEC CARBA NP test kit for detection of Carbapenemase Producing Gram Negative Bacteria.

Evaluation of the RAPIDEC CARBA NP, the Rapid CARB Screen and the Carba NP test for biochemical.
Dortet L, et al.
*JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY* 2015;70:3014-3022.

Evaluation of the RAPIDEC CARBA NP test for the detection of Carbapenemases in *Enterobacteriaceae*.
Hombach M, et al.

RAPIDEC CARBA NP test for rapid detection of carbapenemase producers.
Poirel L, et al.

POSTERS

**ECCMID 2016** / Amsterdam (The Netherlands)
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)
Fast carbapenemase detection by the RAPIDEC CARBA NP
Dévigne L, et al.
A two-centre evaluation of RAPIDEC® CARBA NP for carbapenemase detection in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Kabir MH¹, Meunier D², Hopkins KL², Giske CG³, Woodford N².

(¹) Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.  
(³) Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden.

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 hour 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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(a) Department of Microbiology, GSVM Medical College, Kanpur, India
(b) Department of Anesthesia, GSVM Medical College, Kanpur, India
(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 carbapenem-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.”
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 96.8% (95% CI 91.1%-98.9%) 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).”
Evaluation of the RAPIDEC CARBA NP test for the detection of Carbapenemases in Enterobacteriaceae

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(1) Institut für Medizinische Mikrobiologie, Universität Zürich, Zurich, Switzerland
(2) Viollier AG, Basel, Switzerland

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 was comprised of 51/252 (20.2%) genetically confirmed carbapenemase producers (VIM, KPC, IMI, NDM, GIM, OXA-48). The negative control group was 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 test for rapid detection of carbapenemase producers

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(b) HFR–Hôpital Cantonal, Fribourg, Switzerland

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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1) Scientific Research Institute of Children’s Infections, Saint Petersburg, Russia
2) North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

BACKGROUND
The emergence and rapid dissemination of diverse carbapenemases in Russian hospitals is a major threat for the national health care system. A number of phenotypic and molecular methods have been developed for carbapenemase detection. The aim of the study was to evaluate the performance of the RAPIDEC® CARBA NP (bioMérieux, France) test on clinical isolates of Gram-negative bacteria producing main carbapenemases.

MATERIAL AND METHODS
Non-duplicate clinical isolates of Enterobacteriaceae, Acinetobacter spp. and Pseudomonas aeruginosa characterized phenotypically and genetically for the presence of blaNDM, blaKPC, blaOXA-40-like, blaOXA-23-like, blaVIM, and blaOXA-48 were included in the study. The RAPIDEC® CARBA NP assay was applied to cultures grown overnight on Mueller Hinton E agar (bioMérieux) according to the manufacturer’s protocol. Reactions were read after 30 and 120 minutes of incubation.

K. pneumoniae ST 340 harbouring blaNDM-1 gene, the presence of which was confirmed by PCR and Sanger sequencing, was used for the positive reaction’s control. K. pneumoniae ATCC 700603 was used for the negative reaction’s control.

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-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>
<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>Meropenem</td>
<td>Imipenem</td>
<td></td>
</tr>
<tr>
<td>OXA-48</td>
<td>Enterobacteriaceae (11)</td>
<td>1-32</td>
<td>2-16</td>
<td>+1</td>
</tr>
<tr>
<td>NDM-1</td>
<td>Enterobacteriaceae (13)</td>
<td>8-&gt;64</td>
<td>4-&gt;64</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>A. nosocomialis (2)</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>KPC-2</td>
<td>Enterobacteriaceae (10)</td>
<td>8-&gt;64</td>
<td>4-&gt;64</td>
<td>10</td>
</tr>
<tr>
<td>VIM-type</td>
<td>Enterobacteriaceae (2)</td>
<td>1-8</td>
<td>0.5-16</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa (7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-40, OXA-23</td>
<td>A. baumannii (7)</td>
<td>2-16</td>
<td>2-8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Entero bacteriaceae (29)</td>
<td>0.015-0.12</td>
<td>0.015-0.06</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A. baumannii (10)</td>
<td>0.06-0.25</td>
<td>0.06-0.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa (8)</td>
<td>0.06-0.25</td>
<td>0.06-0.25</td>
<td>0</td>
</tr>
</tbody>
</table>

1(+) - positive result, the colour changed from red to yellow, orange or red-orange
2 (-) - negative result, the colour didn’t change

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.

CONCLUSIONS
RAPIDEC® CARBA NP is suitable for rapid and easy evaluation of carbapenemase activity in Gram-negative bacteria and diagnosis/screening of carbapenemase-producing patients. After the first reading at 30 minutes, the sensitivity is at 65.4%. As some 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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“Attikon” Hospital, National and Kapodistrian University; Laboratory of Clinical Microbiology, Athens, Greece

BACKGROUND
Early detection of carbapenemase-producing (CP) gram-negative pathogens can be crucial for the choice of effective therapy and the outcome of blood-stream 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 carbapenemases from pure cultures of Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa, based on a biochemical method [1, 2]. In order to expand its clinical utility, we applied this test directly to positive blood culture broth.

METHODS

➜ Inoculation of BACTEC Plus Aerobic/F Culture Vials (BD) with 10ml of sterile human blood and 10^3CFU of each isolate

• Thirty CP clinical isolates (6 KPC- and 2 VIM-producing Klebsiella pneumoniae, 17 OXA-type producing A. baumannii, and 5 VIM-type producing P. aeruginosa) and 4 negative controls (E. coli ATCC 25922 and three wild-type clinical isolates).

➜ Incubation in BACTEC 9000 system (BD) until positivity

➜ Centrifugation of 6 ml broth samples of each positive bottle at 160xg for 5 min. Centrifugation of supernatant at 650xg for 10 min.

➜ 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.

RESULTS

Table 1. Performance of the RAPIDEC® CARBA NP test for the detection of carbapenemases without (A) and with (B) enrichment

<table>
<thead>
<tr>
<th>K. pneumoniae</th>
<th>A. baumannii</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>100%</td>
<td>A</td>
</tr>
<tr>
<td>VIM</td>
<td>100%</td>
<td>NA</td>
</tr>
<tr>
<td>OXA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Direct RAPIDEC CARBA NP® was positive for all blood cultures inoculated with K. pneumoniae.

➜ A. baumannii-inoculated blood cultures were negative when tested without enrichment, but two out of four were positive after the enrichment step with TSB.

➜ P. aeruginosa harbouring vials were negative by both methods.

➜ No false-positive results

➜ Hands-on time: 10 min

➜ Turnaround time: 1.5h after blood culture positivity without enrichment and 4.5h with enrichment.

➜ The test requires only standard laboratory equipment.

➜ No specific expertise is needed.

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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R&D Microbiology, bioMérieux SA, La Balme les Grottes, France

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 2359-2364), 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>Nb 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 EB</td>
<td>KPC</td>
<td>E. coli, 4 Enterobacter, 9 K. Pneumoniae, 2 Citrobacter freundii, 1 Proteus mirabilis</td>
</tr>
<tr>
<td>16 EB</td>
<td>NDM-1</td>
<td>E. coli, 3 Enterobacter cloacae, 2 Proteae 4 K. Pneumoniae, 2 Citrobacter</td>
</tr>
<tr>
<td>9 EB</td>
<td>IMP</td>
<td>1 E. coli, 3 Enterobacter cloacae, 3 K. Pneumoniae, 2 Sonella marcescens</td>
</tr>
<tr>
<td>13 EB</td>
<td>VIM</td>
<td>4 E. coli, 3 Enterobacter cloacae, 6 K. Pneumoniae</td>
</tr>
<tr>
<td>21 EB</td>
<td>OXA-48</td>
<td>3 Enterobacter, 17 K. pneumoniae, 1 Proteus rettgeri</td>
</tr>
<tr>
<td>1 EB</td>
<td>GES-5</td>
<td>1 Enterobacter cloacae</td>
</tr>
<tr>
<td>1 EB</td>
<td>NDM-1/OXA-48</td>
<td>1 Klebsiella pneumoniae</td>
</tr>
<tr>
<td>2 nonEB</td>
<td>GES-5</td>
<td>1 PSE ges-2, 1 PSE ges-5</td>
</tr>
<tr>
<td>12 nonEB</td>
<td>IMP</td>
<td>10 PSE, 2 AcB</td>
</tr>
<tr>
<td>7 nonEB</td>
<td>VIM</td>
<td>7 PSE</td>
</tr>
<tr>
<td>5 nonEB</td>
<td>SPM-1</td>
<td>5 PSE</td>
</tr>
<tr>
<td>2 nonEB</td>
<td>NDM-1</td>
<td>2 AcB</td>
</tr>
<tr>
<td>7 nonEB</td>
<td>OXA</td>
<td>1 AcB oxa-23, 3 AcB oxa-23 associated with oxa-51</td>
</tr>
<tr>
<td>5 nonEB</td>
<td>SPM-1</td>
<td>3 AcB oxa-58 associated with oxa-51</td>
</tr>
</tbody>
</table>

Non CPGNB

<table>
<thead>
<tr>
<th>Nb of strains</th>
<th>Resistance type</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>ampC</td>
<td>20 EB, 2 PSE</td>
</tr>
<tr>
<td>22</td>
<td>ESBL</td>
<td>19 EB, 3 PSE</td>
</tr>
<tr>
<td>13</td>
<td>R due to porin loss</td>
<td>7 E. coli, 2 Enterobacter, 2 K. pneumoniae, 2 PSE</td>
</tr>
<tr>
<td>6</td>
<td>wild strains</td>
<td>3 E. coli, 1 PSE, 2 K. pneumoniae</td>
</tr>
</tbody>
</table>
**RESULTS**

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

<table>
<thead>
<tr>
<th>Total of strains</th>
<th>RAPIDEC®</th>
<th>CNP</th>
<th>MHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>EB KPC</td>
<td>20</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>EB NDM</td>
<td>17</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>EB VM</td>
<td>13</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>EB IMP</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>EB Oxa-48 like</td>
<td>21</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>other EB (GES)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>11</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>24</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Total of CPGNB</td>
<td>116</td>
<td>113</td>
<td>3</td>
</tr>
<tr>
<td>Total of non CPGNB</td>
<td>63</td>
<td>-</td>
<td>63</td>
</tr>
</tbody>
</table>

**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-fermented 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.

Sensitivity of detection of carbapenemase-producing Acinetobacter is 82% for RAPIDEC®, and 54.5% for CNP test implemented with the specific protocol. CNP could not detect AcB-producing OXA carbapenemase (OXA-23 and OXA-58).

For the 63 non-CPGNB, no strain was detected as false positive with the RAPIDEC® and CNP but 6 strains were positives for MHT: 3 carbapenem resistant by porin loss and 3 producing an ESBL.

The specificity of the RAPIDEC® is impacted by the inoculum: false positives results are observed when the inoculum is too low and not correctly adjusted with the opacity control.

**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.

**KEY POINTS**

- The low-cost RAPIDEC® CARBA test shows very good performance and can be easily implemented for routine use in any lab.