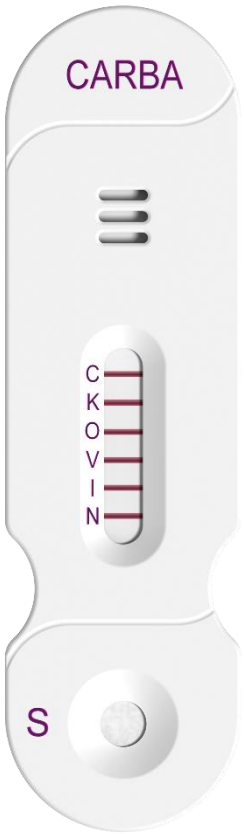
NG·BIOTECH LABORATOIRES

NG-Test® CARBA-5 Literature



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Year: 2017	Country: FR	Sample: Colonies	Objective: Validation

Objectives: The global spread of carbapenemase-producing Enterobacteriaceae represents a substantial challenge in clinical practice and rapid and reliable detection of these organisms is essential. The aim of this study was to develop and validate a lateral flow immunoassay (Carba5) for the detection of the five main carbapenemases (KPC-, NDM-, VIM- and IMP-type and OXA-48-like).

Methods: Carba5 was retrospectively and prospectively evaluated using 296 enterobacterial isolates from agar culture. An isolated colony was suspended in extraction buffer and then loaded on the manufactured Carba5.

Results: All 185 isolates expressing a carbapenemase related to one of the Carba5 targets were correctly and unambiguously detected in <15 min. All other isolates gave negative results except those producing OXA-163 and OXA-405, which are considered low-activity carbapenemases. No cross-reaction was observed with non-targeted carbapenemases, ESBLs, AmpCs or oxacillinases (OXA-1, -2, -9 and -10). Overall, this assay reached 100% sensitivity and 95.3% (retrospectively) to 100% (prospectively) specificity.

Conclusions: Carba5 is efficient, rapid and easy to implement in the routine workflow of a clinical microbiology laboratory for confirmation of the five main carbapenemases encountered in Enterobacteriaceae.

Hopkins KL, Meunier D, Naas T, Volland H, Woodford N. *et al.* Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. J Antimicrob Chemother. 2018;73(12):3523-3526. doi:10.1093/jac/dky342

Year: 2018	Country: UK	Sample: colonies	Objective: Evaluation

Abstract

No single method currently commercialized for detecting acquired carbapenemases offers comprehensive coverage, but local testing is needed to support rapid detection and prompt action. Within the UK and globally most carbapenemase producers harbour one or more of the 'big 5' families: KPC, OXA-48-like, NDM, VIM and IMP. Diagnostic laboratories should therefore consider tests that reliably detect at least four or, preferably, all five of these families.

Here, we evaluated the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, Guipry, France), for detecting the 'big 5' carbapenemases in isolates referred to the UK's national reference laboratory. The NG-TestCARBA5assaywasevaluatedusing197previously characterized bacterial isolates including 177 confirmed carbapenemase producers and 20 carbapenem-resistant but carbapenemase-negative isolates (Table 1). Isolates represented the diversity of carbapenemase genes (but not the prevalence), and host organisms thereof, identified in the UK. One colony of overnight growth harvested from Columbia blood agar plates was tested according tothe manufacturer's instructions. Time until appearance of one or more red lines in the test region of the cassette was recorded by comparison to a line in the control region, but with the final reading performed at 15min as per the manufacturer's instructions. Any discrepancies between the results obtained with the NG-Test CARBA 5 and those obtained with in-house PCRs and/or WGSwereinvestigatedby retesting using the NG-Test CARBA 5 and in-house PCR. Where discrepancies still remained, the carbapenem inactivation method (CIM) was usedtoscreenforcarbapenemaseactivity.

Keywords

Enterobacterales; carbapenem resistance; immunochromatographic assay; positive blood culture

Bodendoerfer E, Keller PM, Mancini S. *et al.* Rapid identification of NDM-, KPC-, IMP-, VIM- and OXA-48-like carbapenemase-producing Enterobacteriales from blood cultures by a multiplex lateral flow immunoassay. J Antimicrob Chemother. 2019;74(6):1749-1751. doi:10.1093/jac/dkz056

Year: 2019	Country: CH	Sample: PBC	Objective: Evaluation

Abstract

In this proof-of-principle study we demonstrated that identification of the 'big five' carbapenemases directly from positive blood cultures is possible using a cost-effective, easy-to-perform 20–40min protocol. However, this study presents some limitations.

Although we included a large number of the most prevalent carbapenemase types in Switzerland, only a few IMP and VIM variants were present. Using the proposed bacterial preparatory method and the Carba-5 test, detection and characterization of the 'big five' carbapenemases in CPE causing BSIs can be achieved with minimal technical expertise in 20–40min. This protocol is faster than any currently available assay and can be implemented in any microbiology laboratory for the detection of CPE BSIs in outbreaks caused by a CPE or in endemic areas, such as Italy, where up to 34% of the Klebsiella pneumoniae causing BSI in 2015 were carbapenem-resistant

Baeza LL, Pfennigwerth N, Greissl C, *et al.* Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm. Clin Microbiol Infect. 2019;25(10):1286.e9-1286.e15. doi:10.1016/j.cmi.2019.03.003

Year: 2019	Country: DE	Sample: Colonies	Objective: Competitor

Objectives: The aim of this study was to evaluate the performance of five different carbapenemase tests and to develop an algorithm which will permit the detection of most common and rare carbapenemases in routine microbiology laboratories.

Methods: The immunochromatographic tests CARBA-5 (NG), RESIST-4 O.K.N.V. (Coris), the colorimetric β-CARBA (BioRad), a newly developed carbapenem-inactivation method (CIM) supplemented with zinc (zCIM), and the Xpert Carba-R (Cepheid) were challenged with a collection of 189 molecularly characterized Enterobacterales isolates, including 146 carbapenemase producers (CPE): VIM (n = 48), OXA-48-like (n = 40), NDM (n = 29), KPC (n = 13), IMI (n = 9), IMP (n = 9), OXA-58 (n = 2), and GES (n = 2).

Results: The overall sensitivity/specificity values for the five carbapenemase detection tests were 84.2% (CI 77.6-89.2%)/100% (CI 91.8-100%) for RESIST-4, 88.2% (CI 82.1-92.4%)/100% (CI 91.8-100%) for CARBA-5, 88.2% (CI 82.1-92.4%)/100% (CI 91.8-100%) for Xpert Carba-R, 73.7% (CI 66.2-80.0%)/100% (CI 93.4-99.0%) for β-CARBA, and 97.4% (CI 87.9-99.6%)/97.7% (CI 87.9-99.6%) for zCIM. The four common carbapenemases (KPC, OXA-48-like, NDM, and VIM) were detected with ≥97.6% sensitivity by all tests except for β-CARBA (76.6% (CI 68.4-83.2%)). IMI and GES were only detected by zCIM (sensitivity 90.9% (CI 62.3-98.4%)). Based on these results a new algorithm was developed, consisting of an immunochromatographic assay as the first test followed by zCIM, which allows detection of 99.3% of all carbapenemases assessed.

Conclusions: Except for β-CARBA, all methods showed excellent sensitivity/specificity for the detection of the four most frequent carbapenemases. With the new algorithm, rare variants can also be detected. It is rapid, simple, and inexpensive and can be performed in any microbiology laboratory, as no PCR equipment is required.

Kieffer N, Poirel L, Nordmann P. *et al.* Rapid immunochromatography-based detection of carbapenemase producers. Infection. 2019;47(4):673-675. doi:10.1007/s15010-019-01326-1

Year: 2019	Country: CH	Sample: Colonies	Objective: Evaluation

Abstract

Objectives: Recent and rapid carbapenemase detection tests are currently available and are based on the biochemical detection of the carbapenem hydrolysis [1, 2]. Recently, an immunochromatographic detection test, the NG-test Carba 5 (NG Biotech, Guipry, France), has been developed. This lateral flow technique allows the detection of the five main carbapenemase types, i.e., KPC, IMP, VIM, NDM, and OXA-48-types (OXA-48, OXA-162, OXA-181, OXA-204, OXA-232, and OXA-244).

Methods: We tested a collection of 73 carbapenem-resistant Gram-negative bacilli. This collection included 45/73 Enterobacteriaceae (Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, and Serratia marcescens), 11/73 Acinetobacter baumannii and 17/73 Pseudomonas aeruginosa.

Results: The NG-test Carba 5 kit allowed the identification of all KPC, OXA-48-types, NDM, VIM and IMP-producers. Noteworthy, this test was able to detect both carbapenemases in the isolates co-producing both NDM-1 and OXA-48-types. Altogether, the values of sensitivity and specificity of this test were 100%. These results are in full agreement with a previous evaluation that has been published since our work was in progress

Conclusions: the NG-test Carba 5 represents a reliable tool to detect the production of the five most common carbapenemase families identified in Gram-negative bacteria and showed a high sensitivity even towards different variants of these enzymes.

Foschi C, Gaibani P, Lombardo D, Re MC, Ambretti S. *et al.* Rectal screening for carbapenemase-producing Enterobacteriaceae: a proposed workflow. J Glob Antimicrob Resist. 2020;21:86-90. doi:10.1016/j.jgar.2019.10.012

Year: 2019	Country: IT	Sample: Colonies	Objective: Evaluation

Objectives: Active screening is a crucial element for the prevention of carbapenemase-producing Enterobacteriaceae (CPE) transmission in healthcare settings. Here we propose a culture-based protocol for rectal swab CPE screening that combines CPE detection with identification of the carbapenemase type.

Methods: The workflow integrates an automatic digital analysis of selective chromogenic media (WASPLab1; Copan), with subsequent rapid tests for the confirmation of carbapenemase production [i.e. detection of Klebsiella pneumoniae carbapenemase (KPC)-specific peak by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) or a multiplex immunochromatographic assay identifying the five commonest carbapenemase types]. To evaluate the performance of this protocol in depth, data for 21 162 rectal swabs submitted for CPE screening to the Microbiology Unit of S. Orsola-Malpighi Hospital (Bologna, Italy) were analysed.

Results: Considering its ability to correctly segregate plates with/without Enterobacteriaceae, WASPLab Image Analysis Software showed globally a sensitivity and specificity of 100% and 79.4%, respectively. Of the plates with bacterial growth (n = 901), 693 (76.9%) were found to be positive for CPE by MALDI-TOF/ MS (KPC-specific peak for K. pneumoniae) or by immunochromatographic assay. Only 2.8% (16/570) of KPC-positive K. pneumoniae strains were missed by the specific MALDI-TOF/MS algorithm, being detected by the immunochromatographic assay. The mean turnaround time needed from sample arrival to the final report ranged between 18 and 24 h, representing a significant time saving compared with manual reading.

Conclusions: This workflow proved to be fast and reliable, being particularly suitable for areas endemic for KPC-producing K. pneumoniae and for high-throughput laboratories.

Takissian J, Bonnin RA, Naas T, Dortet L. *et al.* NG-Test Carba 5 for Rapid Detection of Carbapenemase-Producing Enterobacterales from Positive Blood Cultures. Antimicrob Agents Chemother. 2019;63(5):e00011-19. Published 2019 Apr 25. doi:10.1128/AAC.00011-19

Year: 2019	Country: FR	Sample: PBC	Objective: Evaluation

Abstract

The immunochromatographic assay, NG-test Carba 5 (NG Biotech), has been evaluated for detection of carbapenemase-producing Enterobacterales (CPE) from spiked blood cultures (n = 205). It detected and discriminated in less than 30 minutes KPC, IMP, VIM, NDM, and OXA-48-like producers with a sensitivity and specificity of 97.7% and 96.1%, respectively. Thus, it might help the rapid optimization of treatment of bloodstream infections due to CPE

Keywords

IMP; KPC; NDM; OXA-48-like; VIM; immunochromatography; rapid diagnostic; septicemia.

Giordano L, Fiori B, D'Inzeo T, *et al.* Simplified Testing Method for Direct Detection of Carbapenemase-Producing Organisms from Positive Blood Cultures Using the NG-Test Carba 5 Assay. Antimicrob Agents Chemother. 2019;63(7):e00550-19. Published 2019 Jun 24. doi:10.1128/AAC.00550-19

Year: 2019	Country: IT	Sample: PBC	Objective: Evaluation

Abstract

We directly tested 484 organisms from clinical (n = 310) and simulated (n = 174) positive blood cultures using the NG-Test Carba 5 assay for carbapenemase-producing Enterobacterales detection. The assay identified all but 4 of the KPC (170/171), OXA-48-like (22/22), VIM (19/21), and NDM (14/15) producers with no false positives. Among the clinical Klebsiella pneumoniae organisms tested, 122 of 123 KPC, 1 of 1 OXA-48-like, and 1 of 2 VIM producers were detected by the assay. Some VIM and NDM producers yielded scant but still-readable bands with the assay. No organisms produced the IMPs that the assay was designed to detect.

Keywords

Enterobacterales; carbapenem resistance; immunochromatographic assay; positive blood culture

P1253 A prospective multi-centre evaluation of the NG-test Carba5, a multiplex immunochromatographic assay for the rapid detection of carbapenemase producing Enterobacteriaceae in culture. ECCMID 2019

Year: 2019	Country: FR	Sample: colonies	Objective: Evaluation

Background: The spread of carbapenemase-producing Enterobacterales (CPE) is a public health concern. Rapid detection and identification of CPE is essential to prevent further spread and inform appropriate antimicrobial therapy. During a prospective multi-centre study, we have evaluated the NG-test Carba5 (NGBiotech), a multiplex Lateral Flow ImmunoAssay (LFIA) allowing the detection of NDM, OXA-48-, KPC-, VIM- and IMP-like carbapenemases from bacterial culture in less than 15 minutes.

Methods: The NG-test Carba5 (NG Biotech) was used to prospectively screen isolates sent to three national reference centres (Belgium, UK, France), a regional referral laboratory (Andalusia, Spain), and a clinical microbiology laboratory (Careggi University Hospital, Florence, Italy) for CPE detection. The NG-test Carba5 was used as recommended by the manufacturer in parallel with the local workflow in place for the detection of CPE. The time to a positive result was recorded.

Results: A total of 1095 isolates was tested between February - October 2018. KPC (n=151; 62% were from Italy), OXA-48-like (n=231; 43%, 26%, 21% came from France, Belgium and UK, respectively), NDM (n=119; 52% and 36% were from UK and France, respectively), VIM (n=94; 61% were from Spain), IMP (n=28; 79% were from Spain) and multiple carbapenemase producers (n=26) were all detected in a time-to positivity average of 2-3 minutes (Table 1). Only 3/652 CPE (IMI-1, OXA-427 and OXA-23) were not detected, illustrating that the NG-test Carba5 was able to detect 99.5% of CPE circulating in the countries involved in the study. Of note, the NG-test Carba5 detected 12 IMP-8-positive isolates not detected by the Xpert® Carba-R assay (Cepheid).

Conclusions: The NG-test Carba5 is able to detect the 'big 5' carbapenemase families on their own or in combination with other carbapenemases. The overall sensitivity and specificity were nearly 100%. It requires minimum hands-on-time (<1 min), is easy to implement and has a time-to-positivity of less than 3 mins, in most of the cases. This tool is critical for implementing rapid infection control measures and is also relevant in areas with a high prevalence of NDM-, OXA-48-, KPC-, VIM- or IMP-like producers to discriminate between the carbapenemase families, especially with novel avibactam-based treatments.

Volland, Hervé *et al*. "Improvement of the Immunochromatographic NG-Test Carba 5 Assay for the Detection of IMP Variants Previously Undetected." *Antimicrobial agents and chemotherapy* vol. 64,1 e01940-19. 20 Dec. 2019, doi:10.1128/AAC.01940-19

Year: 2019	Country: FR	Sample: colonies	Objective: Evaluation

Abstract

Here, we evaluated the immunochromatographic assay NG-Test Carba 5v2 (NG-Biotech), with improved IMP variant detection on 31 IMP producers, representing the different branches of the IMP phylogeny, including 32 OXA-48, 19 KPC, 12 VIM, 14 NDM, and 13 multiple carbapenemase producers (CPs), 13 CPs that were not targeted, and 13 carbapenemase-negative isolates. All tested IMP variants were accurately detected without impairing detection of the other carbapenemases. Thus, NG-Test Carba 5v2 is now well adapted to countries with high IMP prevalence and to the epidemiology of CP-Pseudomonas aeruginosa, where IMPs are most frequently detected.

Ben-Haim O, Azrad M, Saleh N, Tkhawkho L, Peretz A. *et al.* Evaluation of the NG-Test CARBA 5 Kit for Rapid Detection of Carbapenemase Resistant Enterobacteriaceae. Lab Med. 2021;52(4):375-380. doi:10.1093/labmed/lmaa084

Year: 2020	Country: IL	Sample: colonies	Objective: Competitor

Objective: We evaluated NG-Test CARBA 5, a new phenotypic carbapenemase detection assay, and compared it to the routine Xpert CARBA-R polymerase chain reaction assay. Furthermore, we tested the kit's performance after bacterial growth on 4 different solid media.

Methods: Seventy carbapenem resistant Enterobacteriaceae (CRE) isolates (60 were carbapenemase producers) were collected at the Poriya Baruch Padeh Medical Center. All isolates were grown on 4 types of agar media-BD BBL CHROMagar carbapenem resistant Enterobacteriaceae, BD CHROMagar Orientation, BD MacConkey II agar, and BD Trypticase Soy Agar II with 5% sheep blood-and were then subjected to NG-Test CARBA 5 kit analysis.

Results: The NG-Test CARBA 5 specificity was 100% for all 4 media. However, the sensitivity was higher when bacteria were grown on TSA with 5% sheep blood (98.3%) as compared with the Orientation medium (88.3%), the CPE medium (84.7%), and the MacConkey medium (83.6%). In addition, some of the carbapenemase mechanisms such as Verona Integron-Mediated Metallo-β-lactamase were detected with low agreement levels in specific media but higher agreement levels in the other media.

Conclusion: NG-Test CARBA 5 may enable faster detection of carbapenemase producing CRE, which will be of value for treatment adjustment and prevention control. However, the medium type on which the bacteria are grown affects kit sensitivity.

Keywords: NG-Test Carba 5 kit; carbapenem resistant Enterobacteriaceae; carbapenemase producing Enterobacteriaceae; diagnostic accuracy; performance; rapid test

Stokes W, Pitout J, Campbell L, Church D, Gregson D. *et al.* Rapid detection of carbapenemase-producing organisms directly from blood cultures positive for Gram-negative bacilli. Eur J Clin Microbiol Infect Dis. 2021;40(2):381-384. doi:10.1007/s10096-020-04005-4

Year: 2020	Country: CA	Sample: PBC	Objective: Evaluation

Introduction: The rapid detection of carbapenemase-producing organisms (CPOs) directly from blood cultures (BCs) positive for Gram-negative bacilli (GNB) may accelerate the appropriate treatment of at-risk patients.

Objective: To evaluate the performance of two commercial assays in the rapid detection of CPOs directly from BC positive for GNB.

Methods: BC positive for GNB were tested for the presence of CPOs with β CARBA® and NG-Test® CARBA 5. A subset of sterile BC samples was seeded with multidrug-resistant (MDR) GNB. Positive BCs from clinical and seeded samples were tested directly with β CARBA and CARBA 5 from BC pellets.

Results: Sixty-five samples were tested (30 clinical, 35 seeded). β CARBA had a sensitivity, specificity, NPV, and PPV of 100%, 65.7%, 100%, and 71.4%, respectively. CARBA 5 had a sensitivity, specificity, NPV, and PPV of 90.0%, 100%, 92.1%, and 100%. False negatives for CARBA 5 occurred with 1 GES, 1 VIM-1, and 1 IMP-14.

Conclusions: This study demonstrates that the detection of CPOs directly from positive BC can be accurately achieved. β CARBA had excellent sensitivity but suffered from poor specificity. CARBA 5 had good sensitivity and specificity but is unable to detect certain CPOs.

Keywords: Bacteremia; CARBA 5; Carbapenemase-producing organisms; Rapid diagnostics; β CARBA

Bianco G, Boattini M, van Asten SAV, *et al.* RESIST-5 O.O.K.N.V. and NG-Test Carba 5 assays for the rapid detection of carbapenemase-producing Enterobacterales from positive blood cultures: a comparative study. J Hosp Infect. 2020;105(2):162-166. doi:10.1016/j.jhin.2020.03.022

Year: 2020	Country: IT	Importance: 2	Sample: PBC	Objective: Competitor

Introduction:

We prospectively compared the performance of RESIST-5 O.O.K.N.V. and NG-Test Carba 5 assays directly from blood cultures spiked with 130 characterized Enterobacterales isolates. Overall, both assays yielded 100% sensitivity to detect KPC-type carbapenemases and OXA-48-like carbapenemases. Both assays failed to detect KPC-31 and KPC-33, D179Y point mutation variants of KPC-3 and KPC-2, that are deprived of carbapenemase activity and confer resistance to ceftazidime-avibactam. On blood culture bacterial pellets, NDM- and VIM-type carbapenemases were detected in 50.0% and 52.2%, respectively, by RESIST-5 O.O.K.N.V. vs 100% by NG-Test Carba 5. The sensitivity of RESIST-5 O.O.K.N.V. improved to 100% and 95.6%, respectively, by performing the assay on 4-h early subculture.

Keywords: Blood culture; Carbapenemase detection; Ceftazidime-avibactam resistance; D179Y; Enterobacterales; Immunochromatographic assay.

Chan WW, Campbell L, Doyle D, Pitout JD. *et al.* Rapid detection of Enterobacterales that produce carbapenemases [published correction appears in Diagn Microbiol Infect Dis. 2021 Feb;99(2):115278]. Diagn Microbiol Infect Dis. 2020;98(2):115120. doi:10.1016/j.diagmicrobio.2020.115120

Year: 2020	Country: CA	Sample: colonies	Objective: Competitor

Purpose: The rapid detection of carbapenemases among Enterobacterales in clinical laboratories is critical for management of patients, and infection prevention and control efforts.

Methods: A study was designed to evaluate the performances of the RAPIDEC CARBA NP®, β-CARBA®, NG-Test CARBA 5®, modified carbapenem-inactivation method, and EDTA version (eCIM) assays against a global collection of Enterobacterales (n = 216) with diverse carbapenemases.

Results: The RAPIDEC CARBA NP® assay had a sensitivity of 98.6% and specificity of 19.6% and β-CARBA® a sensitivity of 94% and specificity of 97.8%, but showed low sensitivity with Klebsiella Pneumoniae Carbapenemase (KPC)-containing isolates. The NG-Test CARBA 5® had an overall sensitivity of 96.3% and specificity of 100% and failed to detect isolates with blaIMP-13, blaIMP-14. The eCIM gave false- positive results with Oxacillinase (OXA)-48-like enzymes.

Conclusion: The NG-Test CARBA 5® assay was technically simple and provided rapid accurate results on the types of carbapenemases. Such information has potential treatment benefits for patients.

Keywords: Carbapenemases; Enterobacterales; Laboratory detection; Rapid.

Jenkins S, Ledeboer NA, Westblade LF, *et al.* Evaluation of NG-Test Carba 5 for Rapid Phenotypic Detection and Differentiation of Five Common Carbapenemase Families: Results of a Multicenter Clinical Evaluation. J Clin Microbiol. 2020;58(7):e00344-20. Published 2020 Jun 24. doi:10.1128/JCM.00344-20

Year: 2020	Country: US	Sample: colonies	Objective: Evaluation

Introduction NG-Test Carba 5 is a rapid in vitro multiplex immunoassay for the phenotypic detection and differentiation of five common carbapenemase families (KPC, OXA-48-like, VIM, IMP, and NDM) directly from bacterial colonies. The assay is simple to perform and has recently received U.S. Food and Drug Administration clearance.

Methods A method comparison study was performed at geographically diverse medical centers (n = 3) in the United States, where 309 Enterobacterales and Pseudomonas aeruginosa isolates were evaluated by NG-Test Carba 5 (NG Biotech, Guipry, France), the Xpert Carba-R assay (Cepheid, Inc., Sunnyvale, CA), the modified carbapenem inactivation method (mCIM), the EDTA-modified carbapenem inactivation method, and disk diffusion with carbapenems. Colonies from tryptic soy agar with 5% sheep blood (blood agar) and MacConkey agar were tested, and the results were compared to those obtained by a composite reference method. Additionally, a fourth medical center performed a medium comparison study by evaluating the performance characteristics of NG-Test Carba 5 from blood, MacConkey, and Mueller-Hinton agars with 110 isolates of Enterobacterales and P. aeruginosa These results were compared to the expected genotypic and mCIM results.

Results For the multicenter method comparison study, the overall positive percent agreement (PPA) and the overall negative percent agreement (NPA) of NG-Test Carba 5 with the composite reference method were 100% for both blood and MacConkey agars. The medium comparison study at the fourth site showed that the PPA ranged from 98.9% to 100% and that the NPA ranged from 95.2% to 100% for blood, MacConkey, and Mueller-Hinton agars.

Conclusions NG-Test Carba 5 accurately detected and differentiated five common carbapenemase families from Enterobacterales and P. aeruginosa colonies on commonly used agar media. The results of this test will support a streamlined laboratory work flow and will expedite therapeutic and infection control decisions

Keywords: Enterobacterales; NG-Test Carba 5; Pseudomonas aeruginosa; Xpert Carba-R; carbapenemase; eCIM; mCIM.

Carvalho I, Alonso CA, Silva V, *et al.* Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae Isolated from Healthy and Sick Dogs in Portugal. Microb Drug Resist. 2020;26(6):709-715. doi:10.1089/mdr.2019.0205

Year: 2020	Country: PT	Sample: colonies	Objective: Epidemiology

Introduction

Extended-spectrum beta-lactamase (ESBL)- and carbapenemase (CP)-producing Klebsiella pneumoniae isolates are a public health concern at clinical level, mainly in Southern European countries. However, there are scarce data on the role of companion animals in the emergence of resistance to clinically relevant antibiotics. Therefore, our study aimed to determine the presence of K. pneumoniae with relevant beta-lactamases in fecal samples from healthy dogs (kennel and house dogs) and sick dogs in seven different hospitals in Portugal. Fecal samples from 125 healthy dogs and 231 sick dogs (one per animal) were collected during April-August 2017. Samples were screened on MacConkey agar supplemented with meropenem, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) was used for K. pneumoniae identification. Genotypic detection of ESBLs or CPs was carried out by PCR/sequencing. Moreover, the presence of other antimicrobial resistance genes and multilocus sequence typing was tested by PCR/sequencing. K. pneumoniae isolates were obtained from 16 tested samples (4.4%), and 3 of them were ertapenem and/or meropenem intermediate/resistant (all of them imipenem susceptible and negative for CP genes). Fifteen K. pneumoniae isolates were ESBL producers, and they carried the following beta-lactamase genes: blaCTX-M-15+blaSHV-28 (four isolates, in three cases associated with blaTEM-1), blaCTX-M-15+blaSHV-1 (five isolates, associated with TEM-1 in three cases), and blaSHV-28+blaTEM-1 (six isolates). Three ESBL-producing K. pneumoniae isolates of different origins and beta-lactamase genotypes (CTX-M-15+SHV-28, CTX-M-15+SHV-28+TEM-1, or SHV-28+TEM-1) belonged to the lineage ST307, and one isolate was identified as ST15 (CTX-M-15+SHV-1). These findings highlight that dogs are frequent carriers of ESBL-producing K. pneumonia isolates, harboring mostly genes encoding CTX-M-15 or SHV-28, associated in some cases with the high-risk clones ST307 and ST15.

Keywords: CTX-M-15; Klebsiella pneumoniae; SHV-28; ST15; ST307; dogs.

Bogaerts P, Berger AS, Evrard S, Huang TD. *et al.* Comparison of two multiplex immunochromatographic assays for the rapid detection of major carbapenemases in Enterobacterales. J Antimicrob Chemother. 2020;75(6):1491-1494. doi:10.1093/jac/dkaa043

Year: 2020	Country: BE	Sample: colonies	Objective: Competitor

Introduction

Objectives: Two commercially available lateral flow immunochromatographic assays (ICAs) for detection of the major carbapenemases were prospectively assessed for the detection of carbapenemases in Enterobacterales: RESIST-4 O.K.N.V. (Coris BioConcept) and NG-Test CARBA 5 (NG Biotech).

Methods: These two assays were performed prospectively on consecutive Enterobacterales suspected of producing a carbapenemase that were referred to the Belgian National Reference Center for Monitoring Antimicrobial Resistance in Gram-Negative Bacteria between March and June 2018. The intensity of the band corresponding to a carbapenemase for each test was compared using ImageJ software.

Results: Of the 161 isolates tested, a carbapenemase was detected in 91 (60 OXA-48-like, 15 VIM, 9 KPC, 5 NDM, 1 IMP and 1 IMP + OXA-48); in the remaining 70, no carbapenemases were detected. For both tests, the results were 100% concordant with the results of the PCR-sequencing reference method. Two IMP producers were only detected by NG-Test CARBA 5 as IMP is not targeted by RESIST-4 O.K.N.V. The mean intensity of the OXA-48, VIM and NDM bands displayed by NG-Test CARBA 5 was 3 to 3.7 times higher than for RESIST-4 O.K.N.V., while the KPC band was on average 1.7 times more intense with RESIST-4 O.K.N.V.

Conclusions: RESIST-4 O.K.N.V. and NG-Test CARBA 5 are two efficient assays for identification of the major carbapenemases. NG-Test CARBA 5 offers the advantage of detecting IMP, which remains rare in Western countries

Kanahashi T, Matsumura Y, Yamamoto M, Tanaka M, Nagao M. *et al.* Comparison of the Xpert Carba-R and NG-Test CARBA5 for the detection of carbapenemases in an IMP-type carbapenemase endemic region in Japan. J Infect Chemother. 2021;27(3):503-506. doi:10.1016/j.jiac.2020.11.001

Year: 2020	Country: JP	Sample: colonies	Objective: Competitor

Introduction: The real-time PCR assay Xpert Carba-R and the lateral flow immunoassay NG-Test CARBA5 were developed to detect 5 types of carbapenemase genes (blaIMP, blaKPC, blaVIM, blaOXA-48, and blaNDM).

Methods: We compared the diagnostic performance, turn-around time, and cost of these assays. Carbapenemase genes were defined using the Carba NP test, modified Carbapenem Inactivation Methods (mCIM), multiplex PCR, and whole-genome sequencing. We included clinical Enterobacterales isolates (n = 36) and nonfermenting gram-negative bacilli isolates (n = 17) collected from 16 acute-care hospitals in the Kinki region of Japan.

Results: Twenty-six of these 53 isolates were positive according to both of the Carba NP test and mCIM and, contained the following carbapenemase genes: blaIMP-1 (n = 3), blaIMP-6 (n = 1), blaIMP-19 (n = 12), blaIMP-26 (n = 1), blaIMP-41 (n = 2), blaIMP-66 (n = 2), blaNDM-1 (n = 3), and blaVIM-2 (n = 2). All of the remaining 27 isolates were negative according to the Carba NP test, mCIM, and multiplex PCR. The specificities of both assays were 100%. The sensitivity of the Xpert Carba-R assay was as low as 53.8% and that of the NG-Test CARBA5 was 92.3% because the former failed to detect all isolates with blaIMP-19 (n = 12) and the latter failed to detect isolates with blaIMP-66 (n = 2). Both assays can easily be performed in less than 5 min.

Conclusions: The NG-Test CARBA5 assay was superior with regard to assay time and cost per sample. We propose the use of the NG-Test CARBA5 assay in clinical laboratories where IMP-type carbapenemases are endemic.

Keywords: Carbapenemase; IMP-type; NG-Test CARBA5; Xpert Carba-R.

Ratnayake L, Ang HZ, Ong CH, Chan DSG. *et al.* An optimized algorithm with improved turnaround time for detection of carbapenemase-producing Enterobacterales using the NG Test CARBA 5 in a routine laboratory. J Med Microbiol. 2020;69(2):228-232. doi:10.1099/jmm.0.001132

Year: 2020	Country: SG	Sample: colonies	Objective: Competitor

Introduction: Rapid and reliable detection of carbapenemase-producing Enterobacterales (CPE) from surveillance ultures is critical in supporting a good infection control programme. We implemented a new algorithm for CPE detection incorporating the NG Test CARBA 5 in January 2019.

Aim Our goals were to compare turnaround time (TAT), costs and staff requirements between the old and new algorithm, and to evaluate the performance of the CARBA 5 test directly on colonies grown on CARBA Smart agar.

Methods: We analysed and compared the TAT of CPE surveillance cultures processed using the old and new CPE screening algorithm. The total actual reagent costs and staff requirements for the new CPE algorithm were compared with the estimated costs and staff requirements of the old CPE algorithm.

Results: Of 197 isolates included in the evaluation of the new algorithm, 64 were positive for carbapenemases by both CARBA 5 and Xpert Carba-R assay. Of the 133 that were negative, two were found to harbour NDM and IMI genotypes. Significant improvements in TAT were achieved with 88.7 % of cultures with CPE, reported on the same day as growth was observed on CARBA Smart agar compared to none in the old algorithm. The new algorithm incurred lower costs and, based on our workload, the new algorithm is estimated to save 28.9 man-hours annually.

Conclusions: CARBA 5 performs well on colonies growing on CARBA Smart agar and significant improvements in TAT can be achieved without incurring additional costs or staff requirements.

Keywords: Carbapenemase; NG-Test CARBA5; Xpert Carba-R.

Khalifa Hazim O, Okanda T, Abd El-Hafeez AA, *et al.* Comparative Evaluation of Five Assays for Detection of Carbapenemases with a Proposed Scheme for Their Precise Application. J Mol Diagn. 2020;22(9):1129-1138. doi:10.1016/j.jmoldx.2020.05.012

Year: 2020	Country: JP-US-EG	Sample: colonies	Objective: Competitor

Introduction The escalating problem of the dissemination of carbapenemase-producing bacteria (CPB) has gained worldwide attention. The prompt diagnosis of CPB and precise identification of carbapenemases are imperative to enable specific antibiotic therapy and control the spread of these bacteria.

Methods The present study was designed to assess the performance of five important assays for the detection of carbapenemases. The modified carbapenem inactivation method (mCIM), CARBA-5**, GeneXpert Carba-R, BD MAX Check-Points CPO, and GeneFields CPE** assays were evaluated with an international collection of 159 bacterial isolates, including 93 CPB and 66 non-CPB isolates.

Results The overall accuracy/sensitivity/specificity for carbapenemase detection were 100% (95% CI, 97.7%-100%)/100% (95% CI, 96.1%-100%)/100% (95% CI, 94.6%-100%) for mCIM, 98.7% (95% CI, 95.5%-99.9%)/**97.9% (95% CI, 92.5%-99.7%)/100% (95% CI, 94.6%-100%) for CARBA-5,** 96.9% (95% CI, 92.8%-99%)/95.7% (95% CI, 89.4%-98.8%)/98.5% (95% CI, 91.8%-99.9%) for GeneXpert Carba-R, 94.3% (95% CI, 89.5%-97.4%)/90.3% (95% CI, 82.4%-95.5%)/100% (95% CI, 94.6%-100%) for BD MAX Check-Points CPO, and 86.2% (95% CI, 79.8%-91.1%)/77.4% (95% CI, 67.6%-85.5%)/98.5% (95% CI, 91.8%-100%) for GeneFields CPE.

Conclusions Interestingly, mCIM and CARBA-5 assays showed 100% accuracy/sensitivity/specificity for detection of the target genes. Furthermore, all the other assays showed comparable high accuracy (96.9% to 100%), sensitivity (100%), and specificity (96.4% to 100%) for the detection of the target genes. On the basis of these results, a new scheme was proposed for their efficient application. These results confirmed the high sensitivity of the evaluated assays, and the proposed scheme is reliable and improves the overall sensitivity and specificity of the assays

Bonnin RA, Jousset AB, Chiarelli A, *et al.* Emergence of New Non-Clonal Group 258 High-Risk Clones among Klebsiella pneumoniae Carbapenemase-Producing K. pneumoniae Isolates, France. Emerg Infect Dis. 2020;26(6):1212-1220. doi:10.3201/eid2606.191517

Year: 2020	Country: FR	Sample: colonies	Objective: Evaluation

Abstract:

The worldwide spread of Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae (KPC-Kp) isolates was reported to be caused by dissemination of 1 clonal complex (i.e., clonal group [CG] 258, which includes sequence types [STs] 258 and 512). We conducted whole-genome sequencing and epidemiologic analysis of all KPC-Kp isolates in France in 2018 and found that new successful high-risk clones of ST147, ST307, ST231, and ST383 are now the main drivers of blaKPC genes. The blaKPC genes were mostly carried by Tn4401a and Tn4401d structures and a new non-Tn4401 element. Our epidemiologic investigations showed that the emergence of these non-CG258 KPC-Kp isolates in France was linked to dissemination of these clones from Portugal. Thus, KPC-Kp epidemiology has changed in Europe, at least in several non-KPC-endemic countries of western Europe, such as France and Portugal, where CG258 is not the most prevalent clone.

Keywords: France; KPC; Klebsiella pneumoniae; ST147; ST307; antimicrobial resistance; bacteria; bacterial infections; carbapenemase; epidemiology; nosocomial infections; whole-genome sequencing.

Gelmez GA, Can B, Hasdemir U, Soyletir G. *et al.* Evaluation of two commercial methods for rapid detection of the carbapenemase-producing Klebsiella pneumoniae [published online ahead of print, 2020 Oct 10]. J Microbiol Methods. 2020;178:106084. doi:10.1016/j.mimet.2020.106084

Year: 2020	Country: TR	Importance: 2	Sample: colonies	Objective: Competitor

Background: In this study, we evaluated the performance of the two commercial methods (Rapidec Carba NP and NG-Test Carba-5) for the rapid detection of carbapenemase-producing Klebsiella pneumoniae.

Methods: A total of 224 Klebsiella pneumoniae strains previously characterized for carbapenemase genes by polymerase chain reaction were included in the study. The strain collection included 30 non-carbapenemase producers, 85 OXA-48-like, 59 NDM, 14 IMP, 7 KPC, 7 VIM, 19 OXA-48-like plus NDM, and 3 KPC plus NDM producers. Rapidec Carba NP and NG-Test Carba 5 was performed according to the manufacturer's instructions.

Results: NG-Test Carba 5 correctly detected all carbapenemase-producing K. pneumoniae, however, Rapidec Carba NP failed to detect 41% of OXA-48-like producers even with extended incubation time. The overall sensitivity and specificity were 81,9% and 100% for Rapidec Carba NP, 100% and 100% for NG-Test Carba 5, respectively.

Conclusions: Both tests seem to be fast and reliable for the detection of carbapenemase-producing K. pneumoniae especially for microbiology laboratories where molecular tests cannot be performed. However, Rapidec Carba NP with weak hydrolysis activity for OXA-48 like might be used in regions where OXA-48 is not prevalent. The additional advantage of NG-Test Carba 5 is that it specifically detects carbapenemases giving way to threat-related infections with an effective drug such as ceftazidime-avibactam or meropenem- vaborbactam.

Keywords: Carbapenemase-producing Klebsiella pneumoniae; NG-test Carba 5; Rapidec Carba NP.

Kon H, Abramov S, Frenk S, *et al.* Multiplex lateral flow immunochromatographic assay is an effective method to detect carbapenemases without risk of OXA-48-like cross reactivity. Ann Clin Microbiol Antimicrob. 2021;20(1):61. Published 2021 Sep 4. doi:10.1186/s12941-021-00469-0

Year: 2020	Country: IL	Sample: colonies	Objective: Evaluation

Background: It is essential to detect carriers of carbapenemase-producing Enterobacterales in order to implement infection control measures. The objectives of this study was to evaluate the NG-Test® CARBA 5 (CARBA 5) assay for detection of five carbapenemases and to assess the cross reactivity of other OXA-type carbapenemases with the OXA-48-like specific antibodies.

Methods: A total of 197 Enterobacterales isolates were tested. To evaluate the cross reactivity, 73 carbapenem-resistant A. baumannii, harboring OXA-type variants, were tested. Polymerase chain reaction (PCR) served as gold standard for carbapenemase identification.

Results: Excellent agreement was found between PCR and CARBA 5, for all but one isolate. The single false positive result (a blaSME positive S. marcescens isolate) was incorrectly positive for blaOXA-48 by CARBA 5. No cross reactivity was observed. The sensitivity and specificity were 100.0% and 98.0%, respectively.

Conclusions: The CARBA 5 assay is highly sensitive and specific and is recommended as a tool for the detection of the main carbapenemases of interest in clinical microbiology laboratories.

Keywords: Carbapenem resistant; Carbapenemase; Cross reactivity; Enterobacterales; NG-Test® CARBA 5 assay; OXA-48-like.

Fahy S, O'Connor JA, O'Brien D, *et al.* Carbapenemase screening in an Irish tertiary referral hospital: Best practice, or can we do better?. Infect Prev Pract. 2020;2(4):100100. Published 2020 Nov 18. doi:10.1016/j.infpip.2020.100100

Year: 2020	Country: IE	Sample: colonies	Objective: Evaluation

Background Carbapenems are a family of end line antibiotics with increasing levels of resistance that are a cause for concern.

Aim: To ascertain whether the CPE screening programme employed in an acute tertiary hospital is fit for purpose.

Methods We outlined the current working algorithm employed using a universal screening programme over a 26-month screening period. Rectal swabs are cultured on arrival. Those with suspicious growth are further investigated using NG-Carba 5 lateral flow tests and Vitek 2.0 sensitivity cards. These practices were compared with NHS guidelines.

Conclusions In all, 53 true positives were detected from 45 patients since the screening was implemented in early 2018 (46 OXA-48, 6 KPC, 1 NDM). As the rate of screening increased, the number of positive screens decreased over time. There were a lot of similarities between the HSE guidelines and the published NHS CPE toolkit. It was evident that there is no standard practice being employed across all hospitals. Comparing the MUH to national guidelines it appears to be quicker and more effective with universal screening in place at reducing the potential contacts and identifying carriers. Cost analysis indicates that the need to confirm all positive strains in a reference lab is costly, unnecessary and time consuming. There are adequate confirmatory tests available in-house for routine positive screens. It was concluded that infection prevention and control are key to identifying and controlling possible outbreaks in a hospital setting.

Keywords: Carbapenemase-producing Klebsiella pneumoniae; NG-test Carba 5; Rapidec Carba NP.

Boattini, Matteo *et al*. "Fast-track identification of CTX-M-extended-spectrum-β-lactamase- and carbapenemase-producing Enterobacterales in bloodstream infections: implications on the likelihood of deduction of antibiotic susceptibility in emergency and internal medicine departments." *European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology* vol. 40,7 (2021): 1495-1501. doi:10.1007/s10096-021-04192-8

Year: 2021	Country: IT	Sample: PBC	Objective: Evaluation

Background: This study aims at presenting a reliable fast-track diagnostics for the detection of CTX-M ESBL- (CTX-M-p) and carbapenemase-producers (CA-p) directly from blood cultures (BCs) of patients with Enterobacterales (EB) bloodstream infections (BSIs) admitted in emergency and internal medicine departments and its contribution in estimation of in vitro antibiotic susceptibility.

Methods: A fast-track workflow including MALDI-TOF species identification and two lateral flow immunochromatographic assays for the detection of CTX-M-p and CA-p directly from BCs was performed in parallel with conventional routine, and results were compared. A total of 236 BCs of patients suffering from EB BSI were included.

Results Accuracy of the fast-track workflow ranged from 99.6 to 100%. Among E. coli isolates, CTX-M-p (20.5%) were susceptible to ceftolozane-tazobactam (C/T, 97%), ceftazidime-avibactam (CZA, 100%), and piperacillin-tazobactam (TZP, 84.8%), whereas CTX-M-and-main-carbapenemases-non-producer (CTX-M-CA-np, 79.5%) isolates were susceptible to all the antibiotics tested. Among K. pneumoniae isolates, CTX-M-p (23.3%) were poorly susceptible to TZP (40%) but widely susceptible to C/T (90%), CZA (100%), and amikacin (90%), whereas CTX-M-CA-np (55.8%) were also susceptible to cefepime. CA-p K. pneumoniae (20.9%) were susceptible to CZA (88.9%). All the species other than E. coli and K. pneumoniae were CTX-M-CA-np and were widely susceptible to the antibiotics tested except for isolates of the inducible and derepressed AmpC- or AmpC/ESBL-p species.

Conclusions Rapid identification of species and phenotype together with knowledge of local epidemiology may be crucial to determine the likelihood of deduction of in vitro antibiotic susceptibility on the same day of positive BC processing.

Keywords: Bloodstream infection. CTX-M. Carbapenemase. Fast microbiology diagnostics. Enterobacterales. Antimicrobial stewardship

Yoon J, Kim CH, Yoon SY, Lim CS, Lee CK. *et al.* Application of a multiplex immunochromatographic assay for rapid identification of carbapenemases in a clinical microbiology laboratory: performance and turn-around-time evaluation of NG-test Carba 5. BMC Microbiol. 2021;21(1):260. Published 2021 Sep 29. doi:10.1186/s12866-021-02309-9

Year: 2021	Country: KR	Sample: colonies	Objective: Competitor

Background Prompt and accurate identification of carbapenemase production is essential for appropriate treatment and infection control. NG-Test Carba 5 (termed herein "Carba 5"; NG Biotech, Guipry, France) is a multiplex immunochromatographic assay for the rapid phenotypic identification of five major carbapenemases (KPC, NDM, VIM, IMP, and OXA-48-like) from bacterial isolates. This study aimed to evaluate the diagnostic performance of Carba 5 and its impact on the turn-around-time in a clinical microbiology laboratory.

Results Carba 5 was retrospectively evaluated using 78 carbapenemase producers and 23 non-carbapenemase producers confirmed by PCR and sequencing. The performance and time required for carbapenemase identification were prospectively evaluated using 47 carbapenem resistant Enterobacteriaceae isolates, and the results were compared to those obtained using Xpert Carba-R (Cepheid, Sunnyvale, CA, USA). For the bacterial isolates included in retrospective and prospective evaluation, the Carba 5 assay correctly identified 147 isolates except one isolate with a sensitivity of 99.13% (95% CI 95.25-99.98%) and specificity of 100% (95% CI 89.42-100%). The Carba 5 assay missed one VIM-1 among 13 VIM producers. The assay showed a sensitivity of 92.31% (95% CI 63.97-99.81%) for detecting VIM and 100% for detecting KPC, NDM, OXA-48-like, and IMP. Compared to the Xpert Carba-R assay, Carba 5 exhibited 100% agreement and was more time-efficient (median time 24 min vs. 1 h 11 min)

Conclusions The Carba 5 assay has potential as an alternative to molecular methods for detecting major carbapenemases from bacterial isolates in a clinical microbiology laboratory. Compared to the Xpert Carba-R, Carba 5 turns out to be more affordable and time-efficient while showing a comparable performance, and may accelerate therapeutic and infection control decisions.

Keywords: Carbapenemase; Microbiology laboratory; NG-test Carba 5; Turn-around-time; Xpert Carba-R.

Bianco G, Boattini M, Iannaccone M, *et al.* Integrating rapid diagnostics in Gram-negative bloodstream infections of patients colonized by carbapenemase-producing Enterobacterales. J Hosp Infect. 2021;110:84-88. doi:10.1016/j.jhin.2021.01.015

Year: 2021	Country: IT	Sample: PBC	Objective: Evaluation

Background We implemented a fast-track diagnostic approach for Gram-negative bloodstram infections (BSIs) among carbapenemase-producing Enterobacterales (CPE) carriers. Within a large cohort of patients with CPE rectal carriage, 18.1% developed Gram-negative BSIs, of which 69.5% were caused by CPE. Direct matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis provided reliable identification in 97% and 53.8% of monomicrobical blood cultures positive to Enterobacterales and non-fermenting Gram-negative species, respectively.

Results Overall, sensitivity and specificity of NG-Test Carba 5 compared with the composite reference method after discrepant analysis were 100%, in polimicrobial blood cultures too.

Conclusions The combined use of direct MALDI-TOF MS and NG-Test Carba 5 assay might be a reliable and cost-effective tool for accelerating the laboratory diagnosis of CPE BSI in cohorts of high-risk patients such as CPE carriers

Keywords: Bloodstream infection; Carbapenemase producing Enterobacterales; MALDI-TOF MS; Rapid diagnostics; Rectal carriage; Surveillance screening.

Han R, Guo Y, Peng M, *et al.* Evaluation of the Immunochromatographic NG-Test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT for Rapid Detection of KPC-, NDM-, IMP-, VIM-type, and OXA-48-like Carbapenemase Among Enterobacterales. Front Microbiol. 2021;11:609856. Published 2021 Jan 15. doi:10.3389/fmicb.2020.609856

Year: 2021	Country: CN	Sample: colonies	Objective: Competitor

Background Enterobacterales are the most common pathogens for nosocomial infections. The emergence and spread of KPC, NDM, and OXA-48-like carbapenemase-producing Enterobacterales with their extensively drug-resistant characteristics have posed great threats to public health. This study aimed to evaluate the performance of NG-test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT for rapid detection of five carbapenemases (KPC, NDM, VIM, IMP, and OXA-48-like) among Enterobacterales.

Methods: A total of 186 carbapenem-resistant Enterobacterales clinical isolates and 29 reference strains were used in this study. Carbapenemase genes were confirmed by PCR and DNA sequencing. The sensitivities and specificities of these assays were calculated utilizing the VassarStats software.

Results For clinical isolates, the NG-test Carba 5 detected KPC, NDM, OXA-48-like, IMP, and VIM in less than 15 min with the sensitivity and specificity of 100% and 100%, respectively. The RESIST-5 O.O.K.N.V. detected KPC, NDM, OXA-48-like, and VIM with the sensitivity and specificity of 99.4 and 100%. The IMP K-SeT detected all of the IMP producers (6/6). For reference strains, the sensitivity and specificity of NG-test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT were all 100 and 100%, respectively.

Conclusions As efficient, rapid, and convenient diagnostic methods, NG-test Carba 5, RESIST-5 O.O.K.N.V., and IMP K-SeT could help to simplify the complex routine workflow for detecting carbapenemases. Rapid and accurate identification of carbapenemase is of significance for both epidemiological and infection control purposes.

Keywords: IMP K-SeT; NG-test Carba 5; RESIST-5 O.O.K.N.V; carbapenem-resistant Enterobacterales; carbapenemase; immunochromatographic assay.

Baer D, Azrad M, Saleh N, Peretz A. *et al.* Detection of Carbapenem-Resistant Enterobacterales in Simulated Blood Culture in 15 Minutes. Life (Basel). 2021;11(2):145. Published 2021 Feb 14. doi:10.3390/life11020145

Year: 2021	Country: IL	Sample: PBC	Objective: Evaluation

Background Bacteremia leading to sepsis and organ dysfunction is a life-threatening situation, leading to death of up to one fourth of the infected individuals around the world. One major challenge in the treatment of sepsis is the rising prevalence of antibiotic resistant bacteria, such as carbapenem-resistant Enterobacterales (CRE). In recent years, several molecular assays have been developed for the detection of CRE mechanisms, enabling rapid results reporting.

Methods: We evaluated the performance of the NG-Test CARBA 5 (NG Biotech) kit in detection of CRE in simulated blood cultures. Carbapenemase-producing (CP) CRE isolates (n = 38) and non-carbapenemase CRE (Non-CP) isolates (n = 10), previously identified using the routine methods practiced at the clinical microbiology laboratory of the Baruch Padeh Medical Center, Israel, were used in this analysis. Variable concentrations of the bacterial isolates were added to a suspension composed of human blood and saline, simulating the composition of a blood culture. Samples were then transferred to an anaerobic blood culture bottle and later tested with the NG-Test CARBA 5 (NG Biotech) kit, that identifies the CRE mechanism within 15 min.

Results The NG-Test CARBA 5 kit correctly identified 43 samples (89.5%). The sensitivity and specificity of the kits were 86.8% and 100%, respectively

Conclusions In conclusion, the NG-Test CARBA 5 kit is a reliable and accessible tool for the rapid diagnosis of CRE bloodstream infections.

Keywords: NG-Test CARBA 5; bacteremia; blood culture; bloodstream infections; carbapenem-resistant Enterobacterales.

Bianco G, Boattini M, Iannaccone M, *et al.* Carbapenemase detection testing in the era of ceftazidime/avibactam-resistant KPC-producing Enterobacterales: A 2-year experience. J Glob Antimicrob Resist. 2021;24:411-414. doi:10.1016/j.jgar.2021.02.008

Year: 2021	Country: IT	Sample: PBC	Objective: Competitor

Objectives: The aim of this study was to investigate the prevalence of ceftazidime/avibactam (CZA) resistance among carbapenemase-producing Enterobacterales (CPE) blood culture isolates as well as the performance of the main carbapenemase phenotypic detection methods to identify KPC variants associated with CZA resistance.

Methods: Non-duplicate CPE strains isolated from blood cultures during 2018-2020 were tested for antimicrobial susceptibility. Molecular testing was used to identify carbapenemase-producers. Strains harbouring blaKPC and with a CZA minimum inhibitory concentration (MIC) ≥8 mg/L were investigated by sequencing. Subsequentially, five phenotypic carbapenemase detection methods were evaluated on these strains, namely the modified carbapenem inactivation method (mCIM), Rapidec® Carba NP, the disk diffusion synergy test, NG-Test CARBA® 5 and RESIST-5 O.O.K.N.V.

Results Overall, the CZA resistance rate was high (13.7%) and remained relevant (5.9%) excluding metallo-β-lactamases-producers. All isolates harbouringblaKPC mutants (n = 8) were associated with reduced carbapenem MICs and negative results by all detection methods based on revelation of enzyme activity. Lateral flow immunoassays failed to detect KPC-31 (n = 4) and KPC-33 (n = 2) but correctly identified KPC-14 (n = 2). Conversely, isolates harbouring wild-type KPC genes (n = 3) were associated with high-level CZA resistance and carbapenem resistance and tested positive by all of the evaluated methods.

Conclusions In the era of CZA-based therapies, molecular blaKPC identification followed by a carbapenem hydrolysis-based phenotypic assay could be the most reasonable diagnostic algorithm to detect all KPC-producers and to identify mutants associated with impaired carbapenemase activity and CZA resistance.

Keywords: Bloodstream infection; Carbapenemase detection; Ceftazidime/avibactam resistance; D179Y; KPC; KPC-14.

Vasilakopoulou A, Karakosta P, Vourli S, Kalogeropoulou E, Pournaras S. *et al.* Detection of KPC, NDM and VIM-Producing Organisms Directly from Rectal Swabs by a Multiplex Lateral Flow Immunoassay. Microorganisms. 2021;9(5):942. Published 2021 Apr 27. doi:10.3390/microorganisms9050942

Year: 2021	Country: GR	Sample: Rectal Swabs	Objective: Evaluation

Objectives: We report a preliminary evaluation of the NG-Test CARBA 5 immunochromatographic assay for detecting carbapenemases directly from rectal swabs on the same day of sampling.

Methods: Thirty fecal swabs were examined for carbapenemase-producing organisms (CPOs) by conventional culture, PCR, and NG-Test CARBA 5. Each sample was tested by the immunochromatographic assay five times, including direct testing and incubation in trypticase soy broth for 1, 2, 3, and 4 h. Twenty patients yielded CPOs by culture. Immunochromatographic and PCR results were concordant and detected the same 25 carbapenemases (11 KPC, 8 VIM, and 6 NDM). In five cases, we detected co-carriage of KPC and VIM.

Results Compared with PCR, the sensitivity of NG-Test CARBA 5 for the detection of KPC, VIM, and NDM was 80% without incubation, 88% with one hour, 92% with two, and 100% with three hours incubation, while specificity was 100% for all time points. All samples containing adequate fecal content were detected by NG-Test CARBA 5 concordantly with PCR, without incubation.

Conclusions NG-Test CARBA 5 is a reliable test that rapidly detects the presence of carbapenemases at the same day of sampling, directly from rectal swabs. It thus provides early information to guide antimicrobial treatment and infection control interventions.

Keywords: carbapenem resistance; carbapenemase detection; carbapenemase-producing organisms; fecal sample; immunochromatography.

Zhu Y, Jia P, Li X, *et al.* Carbapenemase detection by NG-Test CARBA 5-a rapid immunochromatographic assay in carbapenem-resistant Enterobacterales diagnosis. Ann Transl Med. 2021;9(9):769. doi:10.21037/atm-20-8216

Year: 2021	Country: CN	Sample: Colonies	Objective: Evaluation

Background: The global spread of carbapenem-resistant Enterobacterales (CRE) represents a serious public health concern as these organisms are associated with limited treatment options, high mortality rate and rapid transmissibility. The identification of carbapenemase remains a challenge in microbiological laboratories as no single method is perfect when considering cost, carbapenemase coverage, accuracy, handling complexity and TATs together.

Methods: NG-Test CARBA 5 assay and modified carbapenem inactivation method in conjunction with EDTA carbapenem inactivation method (mCIM/eCIM) were challenged with a collection of 299 molecularly characterized CRE isolates in China in order to evaluate the performance in detecting five major carbapenemases (bla KPC, bla NDM, bla VIM, bla IMP, and bla OXA-48) among Enterobacterales.

Results: NG-Test CARBA 5 detected all KPC-, NDM-, VIM- and OXA-48-producing isolates perfectly with a weak false-positive signal for NDM in an IMP-4 producer, which makes the specificity for NDM decreases to 99.6%. The overall specificity/sensitivity were 99.9%/100% for NG-Test CARBA 5. mCIM/eCIM achieved high specificity of 100%/100% and sensitivity of 99.6%/97.4%, with one S. marcescens isolate harboring VIM-2 undetected.

Conclusions: Both NG-Test CARBA 5 and mCIM/eCIM showed excellent results in the tested carbapenemase (bla KPC, bla NDM, bla VIM, bla IMP, and bla OXA-48) detection compared with molecular genotypic test. As every assay has its own limitations, suitable methods should be combined for the establishment of the CRE diagnostic pathways.

Keywords: Carbapenemase-resistant Enterobacterales (CRE); EDTA carbapenem inactivation method (eCIM); NG-Test CARBA 5; modified carbapenem inactivation method (mCIM)

Lee S, Hur KH, Chung Y, *et al.* Evaluation of Two Commercial Kits for Rapid Detection and Typing of Carbapenemase in Carbapenem-Resistant Enterobacterales. Ann Clin Microbiol. 2021;24(2):45-53.

Year: 2021	Country: KR	Sample: Colonies	Objective: Competitor

Background: Rapid detection of carbapenemase-producing Enterobacterales (CPE) is desirable to guide antimicrobial therapy and infection control. The NG-Test Carba5 (Carba5;NG Biotech, France) rapid multiplex lateral flow immunoassay and BD MAX Check-Points CPO Assay (CPO; BD Diagnostic Systems, USA) fully automated real-time PCR assay were evaluated for the detection of KPC, NDM, VIM, IMP, and OXA-48-like group in a culture colony compared to genotyping using conventional PCR

Methods: Among the clinical isolates of carbapenem-resistant Enterobacterales (CRE) collected from 2013 to 2019, up to 20 isolates for each carbapenemase type, and approximately 60 carbapenemase-negative CRE were enrolled. Genotyping of carbapenemases were performed using single-target PCR for KPC, NDM, and OXA-48-like group and the multiplex PCR for VIM, IMP, GIM, SIM, and SPM. All isolates were tested with Carba5 and CPO. The discrepant results were resolved by single-target specific conventional PCR or GeneXpert Carba-R Assay (Carba-R; Cepheid, USA).

Results: Of 147 CREs, 82 were CPE (55.8%) including 20 KPC, 22 NDM, 17 VIM, three IMP, and 13 OXA-48-like group, and seven double carbapenemase-positive (three KPC/VIM, two NDM/ VIM, one KPC/NDM, and one NDM/OXA-48-like group) isolates. Carba5 and CPO detected all CPE correctly along with two more IMP-producing CPE. The sensitivity and specificity of both kits were equally 100% and 97%. Two false IMP-positives were confirmed IMP-positive with Carba-R and IMP-specific single-target PCR.

Conclusions: Carba5 and CPO reliably detect and differentiate five common carbapenemases in cultured colonies. Carba5, faster and simpler, is preferred as a spot test.

Keywords: Carbapenemase; Enterobacterales; Genotype; Immunoassay; PCR

Kriger O, Shatzman-Steuerman R, Smollan G, Belausov N, Sarkisian G, Amit S. *et al.* Rapid Detection of Carbapenemase-producing Enterobacteriaceae From Blood Culture Bottles of Known CPE Carriers: Real-world Experience. Pediatr Infect Dis J. 2022;41(1):45-47. doi:10.1097/INF.0000000000003311

Year: 2021	Country: IL	Sample: PBC	Objective: Evaluation

Abstract

We used a rapid antigen test for the detection of carbapenemases directly from positive blood culture bottles of pediatric hemato-oncologic patients, known carriers of carbapenemase-producing enterobacteriaceae. Resistance mechanism was detected within 15 minutes of observing Gram-negative bacilli from a positive bottle, leading to treatment modification. This simple-to-use, inexpensive assay shortens the interval between empiric to tailored antimicrobial therapy.

Keshta AS, Elamin N, Hasan MR, *et al.* Evaluation of Rapid Immunochromatographic Tests for the Direct Detection of Extended Spectrum Beta-Lactamases and Carbapenemases in Enterobacterales Isolated from Positive Blood Cultures. Microbiol Spectr. 2021;9(3):e0078521. doi:10.1128/Spectrum.00785-21

Year: 2021	Country: QA	Sample: PBC	Objective: Evaluation

Background: NG-Test CTX-M MULTI and NG-Test Carba 5 (NG Biotech) are two rapid in vitro immunochromatographic assays that are widely used for the detection of the most common extended spectrum beta-lactamases (ESBL) and carbapenemases in Enterobacterales. ESBL and carbapenemases are leading causes of morbidity and mortality worldwide and their rapid detection from positive blood cultures is crucial for early initiation of effective antimicrobial therapy in bloodstream infections (BSI) involving antibiotic-resistant organisms.

Methods: In this study, we developed a rapid workflow for positive blood cultures for direct identification of Enterobacterales by MALDI-TOF mass-spectrometry, followed by detection of ESBL and carbapenemases using NG-Test CTX-M MULTI and NG-Test Carba 5 (NG Biotech). The workflow was evaluated using Enterobacterales isolates (n = 114), primarily Klebsiella species (n = 50) and Escherichia coli (n = 40).

Results: Compared to the standard testing approach in our institution using BD Phoenix, our new testing approach demonstrates 100% sensitivity and specificity for organism identification and detection of ESBL and carbapenemases.

Conclusions: Implementation of a rapid workflow in diagnostic microbiology laboratories will enable more effective antimicrobial management of patients with BSI due to ESBL- and carbapenemase-producing Enterobacterales.

IMPORTANCE The incidence of bloodstream infections (BSI) with extended spectrum beta-lactamase (ESBL) producing and carbapenemase producing Enterobacterales (CPE) is increasing at an alarming rate, for which only limited therapeutic options remain available. Rapid identification of these bacteria along with their antibiotic resistance mechanisms in positive blood cultures with Gram-negative bacteria will allow for early initiation of effective therapy and limit the overuse of broad-spectrum antibiotics in BSI (1). In this study we evaluated a combined approach of testing positive blood cultures directly, using MALDI-TOF MS followed by rapid immunochromatographic tests, for the detection of ESBLs and CPEs. Our approach demonstrates 100% sensitivity and specificity for the identification of Enterobacterales and detection of ESBLs and CPEs in positive blood culture with a turnaround time (TAT) of ≤60 min compared to a TAT of 48 h required by conventional culture and susceptibility testing methods.

Keywords: CPO; CTX-M; ESBL; NG-Test CARBA 5.

Huang YT, Kuo YW, Lee NY, *et al.* Evaluating NG-Test CARBA 5 Multiplex Immunochromatographic and Cepheid Xpert CARBA-R Assays among Carbapenem-Resistant Enterobacterales Isolates Associated with Bloodstream Infection. Microbiol Spectr. 2022;10(1):e0172821. doi:10.1128/spectrum.01728-21

Year: 2022	Country: TW	Sample: Colonies	Objective: Competitor

Background: Decreased susceptibility to carbapenems in Enterobacterales is an emerging concern. Conventional methods with short turnaround times are crucial for therapeutic decisions and infection control.

Methods: In the current study, we used the Xpert CARBA-R (Cepheid, Sunnyvale, CA, USA) and the NG-Test CARBA 5 (NG Biotech, Guipry, France) assays for carbapenemase detection in 214 carbapenem-resistant Enterobacterales (CRE) blood isolates. We used the modified carbapenem inactivation method, conventional PCR, and sequencing to determine the production of five common carbapenemase families and their subtypes. We performed wzc-genotyping for all CR-Klebsiella pneumoniae (CRKP) and multilocus sequence typing for all carbapenemase-producing CRE isolates to reveal their genetic relatedness.

Results: The results showed a sensitivity of 99.8% and a specificity of 100% by the Xpert assay, and a sensitivity of 100% and a specificity of 99% by the NG-Test in detecting carbapenemases of 84 CRKP isolates with only one (VIM-1+IMP-8) failure in both tests. For CR-Escherichia coli, four carbapenemase-producing isolates were detected accurately for their subtypes. The two major clones of carbapenemase-producing CRKP isolates in Taiwan were ST11-K47 producing KPC-2 (n = 47) and ST11-K64 producing OXA-48-like (n = 9).

Conclusions: Our results support the use of either test in routine laboratories for the rapid detection of common carbapenemases. Caution should be taken using the Xpert assay in areas with a high prevalence of CRE carrying blaIMP-8.

IMPORTANCE: Carbapenemase-producing Enterobacterales (CPE) are emerging worldwide, causing nosocomial outbreaks and even community-acquired infections since their appearance 2 decades ago. Our previous national surveillance of CPE isolates in Taiwan identified five carbapenemase families (KPC, OXA, NDM, VIM, and IMP) with the KPC-2 and OXA-48-like types predominant. Timely detection and classification of carbapenemases in CPE may be a useful test to guide optimal therapy and infection control. Genetic detection methods using the Xpert CARBA-R assay and the immunochromatographic assay using the NG-Test CARBA 5 have been validated with the advantage of short turnaround time. Our study demonstrated that the NG and Xpert assays are convenient methods to accurately identify carbapenemases in carbapenem-resistant Klebsiella pneumoniae and carbapenem-resistant Escherichia coli blood isolates. Detecting IMP variants remains challenging, and the results of Xpert CARBA-R assay should be carefully interpreted.

Keywords: NG-Test CARBA 5; Xpert; Xpert CARBA-R; carbapenem-resistant Enterobacterales; multilocus sequence; wzc-genotyping.

Levi G, Lurie-Weinberger M, Keren-Paz A, Andremont AO, Schwartz D, Carmeli Y. *et al.* Unraveling the Diversity of Co-Colonization by CPE. Microorganisms. 2022;10(7):1292. Published 2022 Jun 25. doi:10.3390/microorganisms10071292

Year: 2022	Country: IL	Sample: Colonies	Objective: Epidemiology

Abstract:

Antibiotic-resistant bacteria, and more specifically, carbapenem-producing Enterobacterales (CPE) strains, are increasing worldwide. Despite their growing prevalence, in most high-income countries, the detection of CPE is still considered a low-frequency event. Sporadically, patients co-colonized with distinct CPE strains and/or different carbapenemase enzymes are detected. In this paper, we present three cases that illustrate the underlying mechanisms of co-colonization, focusing on horizontal gene transfer (HGT) and patient-to-patient transmission. We also demonstrate the diversity of CPE species and discuss the potential consequences of co-colonization.

Keywords: CPE; CRE; HGT; co-colonization.

Trusca BS, Ianculescu E, Marutescu L, *et al.* Comparative Performance of Two Immunochromatographic Tests for the Rapid Detection of PCR Confirmed, Carbapenemase Producing Enterobacterales Biointerface Research in Applied Chemistry. doi:10.33263/BRIAC134.322

Year: 2022	Country: RO	Sample: Colonies	Objective: Competitor

Background: The Gram-negative bacilli (GNB) tend to dominate the infectious pathology, often due to multidrug-resistant (MDR) strains and evolving with severe, complicated, and difficult-to-treat clinical forms. This study aimed to investigate by phenotypic and genotypic assays a representative set of carbapenem-resistant GNB strains to evaluate their contribution to appropriate epidemiological surveillance and therapy of associated infections

Methods: A number of 70 Enterobacterales MDR bacterial strains were consecutively isolated from patients with different infections (79 %) and carriers (rectal portages, 21 %) hospitalized at the Fundeni Clinical Institute from November 2017 - April 2018. The strains, previously characterized by PCR, were investigated comparatively by two immunochromatographic tests, NG-Test Carba 5 and RESIST-3 O.K.N., able to detect KPC, OXA-48 like NDM, VIM, IMP, and OXA-48 like, KPC, NDM, respectively

Results: KPC was the main carbapenemase detected (37 %), followed by OXA-48 (30 %). Both rapid immunochromatographic tests demonstrated high sensitivity and specificity, the results being 100 % concordant with the results of the PCR method.

Conclusions: The immunochromatographic assay is, therefore, a cheap and reliable method for the rapid detection, within 15 minutes, of carbapenemase-producing strains. Rapid and accurate identification of carbapenemases is significant for clinical and epidemiological purposes, infection control, and antimicrobial therapy's effectiveness.

Keywords: carbapenemase-resistant Enterobacterales; NG-Test Carba 5; RESIST-3 0.K.N; immunochromatographic.

Saito K, Mizuno S, Nakano R, *et al.* Evaluation of NG-Test CARBA 5 for the detection of carbapenemase-producing Gram-negative bacilli. J Med Microbiol. 2022;71(6): 10.1099/jmm.0.001557. doi:10.1099/jmm.0.001557

Year: 2022	Country: JP	Sample: Colonies	Objective: Evaluation

Background: Carbapenemase-producing Enterobacterales (CPE) pose one of the most serious antimicrobial resistance threats to public health worldwide. The outcome of CPE infection differs depending on the resistance mechanism. Therefore, rapid detection of CPE infection is essential for optimizing patient management. The carbapenem inactivation method (CIM) and modified CIM (mCIM) are standard methods for detecting CPE, but they usually require 24 h to generate results. Recently, an immunochromatographic assay, NG-Test CARBA 5, has become commercially available. It detects the five most common carbapenemase producers (KPC, IMP, NDM, VIM, and OXA-48) rapidly and accurately.

Methods: We aimed to evaluate the diagnostic accuracy of NG-Test CARBA 5 for detecting carbapenemase-producing Gram-negative bacilli (CPGNB). We used 116 carbapenemase-producing strains and 48 non-carbapenemase-producing strains. Of the 116 carbapenemase-producing strains, 107 harboured genes for at least one of the five most common carbapenemases, KPC, IMP, NDM, VIM, and OXA-48-like. Forty-eight non-carbapenemase-producing strains, including carbapenem-resistant Enterobacterales, harboured genes for extended-spectrum β-lactamases (CTX-M groups [n=25] and SHV groups [n=2]) or plasmid-mediated AmpC β-lactamases (DHA [n=3], CMY-2 [n=2], and CFE-1 [n=1]). Antimicrobial susceptibility was tested using the agar dilution method, according to the Clinical and Laboratory Standards Institute guidelines.

Results: Of the 116 carbapenemase-producing strains, 79 were resistant to at least meropenem or imipenem. The sensitivity and specificity of the NG-Test CARBA 5 for the strains were 99.1 % (106 strains positive for 107 strains of the five most common carbapenemase producers) and 100 % (60 strains negative for other types of CPGNB [n=10] and non-CPGNB strains [n=48]), respectively. The carbapenemase-producing strain with a false-negative result produced IMP-66.

Conclusions: The NG-Test CARBA 5 had high sensitivity and specificity for detecting carbapenemase-producing strains.

Keywords: Gram-negative bacilli; antimicrobial resistance; carbapenemase; carbapenemase-producing Enterobacterales; diagnostic accuracy; immunochromatography.

Lobo da Costa J, Karla de Carvalho M, Mendes Nunes V, *et al.* AVALIAÇÃO DO TESTE IMUNOCROMATÁTICO CARBA 5 NG PARA IDENTIFICAÇÃO RÁPIDA DE CARBAPENEMASES EM ISOLADOS CLÍNICOS DE PSEUDOMONAS SP. Brazilian Journal of Infectious Diseases. 2022;26(1): 10.1016/j.bjid.2021.101997 doi.org/10.1016/j.bjid.2021.101997

Year: 2022	Country: BR	Sample: Colonies	Objective: Evaluation

Objective: Pseudomonas sp. é um gênero bacteriano caracterizado como bacilos gram-negativos oportunistas, cuja principal espécie é Pseudomonas aeruginosa. Atualmente as infecções por este microrganismo tem gerado grande preocupação devido ao desenvolvimento de cepas multidroga resistentes (MDR) e extensivamente droga resistentes (XDR), limitando as opções terapêuticas disponíveis, principalmente pela produção das enzimas carbapenemases. Diante disso, o objetivo deste trabalho foi realizar a detecção das enzimas (NDM, VIM, IMP e KPC) em isolados clínicos de Pseudomonas sp. resistentes aos carbapenêmicos oriundos de pacientes internados num hospital terciário de Recife-PE.

Methods: Foram analisados 32 isolados clínicos de Pseudomonas sp. resistentes aos carbapenêmicos, cuja identificação se deu através do maldi tof MS Bruker e o teste de sensibilidade foi realizado através do Phoenix BD. Estes isolados foram submetidos ao teste imunocromatográfico Carba 5 NG. Um teste rápido para a detecção de carbapenemases (KPC, OXA, VIM, IMP e NDM) através das colônias bacterianas de cultura. Os testes foram realizados seguindo as recomendações do fabricante.

Results: Dos 32 isolados analisados, 31 foram identificados como P. aeruginosa, e um isolado foi identificado como Pseudomonas stutzeri. Entre os isolados de P. aeruginosa, a maioria foram provenientes do trato respiratório, 22 amostras, sendo 18 de aspirado traqueal, três de lavado bronco alveolar e uma de escarro. O isolado de P. stutzeri foi proveniente de amostra de aspirado traqueal. Os principais setores de internamento dos pacientes infectados por estas bactérias foram as enfermarias e as Unidades de Terapia Intensiva (UTIs), respectivamente. Em relação aos mecanismos de resistência detectados nestes isolados, entre os 31 isolados de P. aeruginosa, 12 foram positivos para a enzima KPC, nove positivos para VIM e 10 não apresentaram nenhuma das enzimas pesquisadas. Enquanto isso, o isolado de P. stutzeri foi positivo para enzima IMP.

Conclusions: Embora o teste Carba 5 NG apresente 100% de sensibilidade e especificidade para detecção de carbapenemases nas Enterobacteriales, poucos estudos avaliaram a eficácia deste teste em Pseudomonas sp. A detecção rápida destas enzimas nesses isolados é de fundamental importância para direcionar a terapia antimicrobiana adequada, bem como para traçar medidas para interromper a cadeia de disseminação destes microrganismos portadores de mecanismos de resistência.

Nishida S, Ihashi Y, Yoshino Y, Ono Y. *et al.* Evaluation of an immunological assay for the identification of multiple carbapenemase-producing Gram-negative bacteria. Pathology. 2022;54(7):917-921. doi:10.1016/j.pathol.2022.05.007

Year: 2022	Country: JP	Sample: Colonies	Objective: Competitor

Background: Carbapenemase-producing Gram-negative organisms (CPOs) frequently gain multidrug-resistant phenotypes and thereby limit the therapeutic options available. Colonisation and infection with CPOs are critical risks for mortality in clinical settings, especially in critical care medicine. Carbapenemase genes on plasmids have transferred to many Gram-negative species, and these species have spread, leading to global concern regarding antimicrobial resistance. A molecular rapid diagnostic test (mRDT) for CPOs is urgently required in critical care medicine.

Methods: Here, we evaluated a rapid lateral flow immunoassay (LFIA) for CPOs isolated from patients at university hospitals, including intensive care units, and compared the results with those obtained using the multiplex polymerase chain reaction (PCR) method. NG-test CARBA 5 detected multiple carbapenemases, KPC, OXA-48, NDM, VIM, and IMP variants expressed in clinical isolates. Quick Chaser IMP detected IMP variants.

Results: The LFIAs exhibited 100% sensitivity and specificity relative to clinical isolates on agar plates. By contrast, the multiplex PCR method exhibited a limited ability to detect IMP-7-producing isolates not belonging to the IMP1 group, which resulted in 97% sensitivity and 100% specificity for IMP-producing isolates.

Conclusions: Our results demonstrate that the LFIA is a useful mRDT to identify CPOs and has an advantage over the PCR method for both detection time and sensitivity to the IMP groups. LFIA could complement the nucleic acid amplification test used to identify CPOs. In conclusion, we evaluated sensitive and specific LFIAs capable of detecting carbapenemase production in Gram-negative bacteria. We anticipate that LFIAs will become a point-of-care test enabling rapid detection of carbapenemases in hospital settings, particularly in intensive care units.

Keywords: Carbapenemase; Gram-negative bacteria; ICU; LFIA; MDR; POCT; intensive care unit; lateral flow immunoassay; multidrug resistance; multiplex PCR; point-of-care test.

Bernabeu S, Bonnin RA, Dortet L. *et al.* Comparison of three lateral flow immunochromatographic assays for the rapid detection of KPC, NDM, IMP, VIM and OXA-48 carbapenemases in Enterobacterales. J Antimicrob Chemother. 2022;77(11):3198-3205. doi:10.1093/jac/dkac303

Year: 2022	Country: FR	Sample: Colonies	Objective: Competitor

Background:

During the last decade, the emergence and the dissemination of difficult-to-treat bacteria became a major public health issue. Among these difficult-to-treat bacteria, carbapenemase-producing Gram-negatives are often considered the most worrisome. According to the Ambler classification, the most widespread carbapenemases in Enterobacterales belong to Ambler class A (mostly KPC enzymes), MBLs (Ambler class B) of NDM, VIM and IMP type and carbapenem-hydrolysing Ambler class D enzymes of OXA-48 type. During the last 5 years, several new antimicrobial compounds have been marketed for the treatment of difficult-to-treat bacteria, focusing on carbapenemase-producing Enterobacterales (CPE). These new treatments include the association of broad-spectrum β-lactams with new β-lactamase inhibitors (ceftazidime/avibactam, meropenem/vaborbactam, imipenem/relebactam)1,2 and new molecules such as cefiderocol.3 Unfortunately, these new drugs cannot target all CPE. Indeed, the novel β-lactamase inhibitors (avibactam, relebactam and vaborbactam) are not able to inhibit MBLs.

Thus, the early detection of CPE as well as the rapid discrimination of the carbapenemase type is a key point to implement hygiene control measures and to quickly adapt the treatment in case of infection. Among available tests, lateral flow immunochromatography assays (LFIAs) offer numerous advantages, including their easiness, their relative low cost and their rapidity (15 min).

Josa M D, Leal R, Rojas J, *et al.* Comparative Evaluation of Phenotypic Synergy Tests versus RESIST-4 O.K.N.V. and NG Test Carba 5 Lateral Flow Immunoassays for the Detection and Differentiation of Carbapenemases in Enterobacterales and Pseudomonas aeruginosa. Microbiol Spectr. 2022;10(1):e0108021. doi:10.1128/spectrum.01080-21

Year: 2022	Country: CO	Sample: Colonies	Objective: Competitor

Background: The spread of carbapenem-resistant Pseudomonas aeruginosa and carbapenemase-producing Enterobacterales (CPE) has dramatically impacted morbidity and mortality. COVID-19 pandemic has favored the selection of these microorganisms because of the excessive and prolonged use of broad-spectrum antibiotics and the outbreaks related to patient transfer between hospitals and inadequate personal protective equipment. Therefore, early CPE detection is considered essential for their control.

Methods: We aimed to compare conventional phenotypic synergy tests and two lateral flow immunoassays for detecting carbapenemases in Enterobacterales and P. aeruginosa. We analyzed 100 carbapenem-resistant Gram-negative bacilli isolates, 80 Enterobacterales, and 20 P. aeruginosa (86 isolates producing KPC, NDM, OXA-48, IMP, and VIM carbapenemases and 14 non-carbapenemase-producing isolates). We performed a modified Hodge test, boronic acid and ethylenediaminetetraacetic acid (EDTA) synergy tests, and two lateral flow immunoassays: RESIST-4 O.K.N.V. (Coris Bioconcept) and NG Test Carba 5 (NG Biotech). In total, 76 KPC, seven VIM, one NDM, one OXA-48, and one isolate coproducing KPC + NDM enzymes were included.

Results: The concordance of different methods estimated by the Kappa index was 0.432 (standard error: 0.117), thus showing a high variability with the synergy tests with boronic acid and EDTA and reporting 16 false negatives that were detected by the two immunochromatographic methods. Co-production was only detected using immunoassays.

Conclusions: Conventional phenotypic synergy tests with boronic acid and EDTA for detecting carbapenemases are suboptimal, and their routine use should be reconsidered. These tests depend on the degree of enzyme expression and the distance between disks. Lateral flow immunoassay tests are a rapid and cost-effective tool to detect and differentiate carbapenemases, improving clinical outcomes through targeted therapy and promoting infection prevention measures.

IMPORTANCE Infections due to multidrug-resistant pathogens are a growing problem worldwide. The production of carbapenemases in Pseudomonas aeruginosa and Enterobacterales cause a high impact on the mortality of infected patients. Therefore, it is of great importance to have methods that allow the early detection of these multi-resistant microorganisms, achieving the confirmation of the type of carbapenemase present, with high sensitivity and specificity, with the aim of improving epidemiological control, dissemination, the clinical course to through targeted antibiotic therapy and promoting infection control in hospitals.

Keywords: EDTA; boronic acid; carbapenemases; immunoassay; multidrug resistance; synergy test

Zboromyrska Y, Rico V, Pitart C, Fernández-Pittol MJ, Soriano Á, Bosch J. *et al.* Implementation of a New Protocol for Direct Identification from Urine in the Routine Microbiological Diagnosis. Antibiotics (Basel). 2022;11(5):582. Published 2022 Apr 26. doi:10.3390/antibiotics11050582

Year: 2022	Country: ES	Sample: Urines	Objective: Evaluation

Background: The direct identification of uropathogens from urine samples, in combination with the rapid detection of resistance, would allow early adjustment of empirical antimicrobial treatment.

Methods: Two hundred and ninety-eight urine samples processed between 1 June and 31 December 2020, selected with flow cytometry, with direct identification by MALDI-TOF mass spectrometry, and rapid detection of extended-spectrum beta-lactamase (ESBL) and carbapenemases-producing strains by lateral flow were analyzed.

Results The positive predictive value of the direct identification of the 86 samples that met the flow cytometry criterion (>5000 bacteria/µL) was 96.4%. Reliable direct identification was obtained in 14 of the 27 (51.8%) urinary source bacteraemias. There was 100% agreement between the lateral flow and antibiogram in the detection of ESBL and carbapenemases.

Conclusions: The protocol for the direct identification and rapid detection of ESBL and carbapenemases-producing strains from urine samples is a reliable and useful tool.

Keywords: ESBL; MALDI-TOF; carbapenemases; flow cytometry; urinary tract infection; urine.

Comini S, Bianco G, Boattini M, *et al.* Evaluation of a diagnostic algorithm for rapid identification of Gram-negative species and detection of extended-spectrum β-lactamase and carbapenemase directly from blood cultures. J Antimicrob Chemother. 2022;77(10):2632-2641. doi:10.1093/jac/dkac230

Year: 2022	Country: IT	Sample: PBC	Objective: Evaluation

Objectives: To evaluate a rapid diagnostic algorithm based on MALDI-TOF MS, lateral flow immunoassays (LFIAs) and molecular testing performed directly from positive blood cultures (BCs) for Gram-negative species identification and detection of CTX-M extended-spectrum β-lactamases and main carbapenemases.

Methods: Non-duplicate BCs positive to Gram-negative bacteria at microscope examination were subjected to species identification by direct MALDI-TOF MS following recovery of bacterial pellet by Rapid MBT Sepsityper® kit. Subsequently, NG-Test® CARBA 5 and NG-Test® CTX-M MULTI LFIAs were performed according to identified microbial species. Eazyplex® SuperBug CRE molecular assay was performed in cases of NG-Test® CARBA 5 negative results in patients with documented carbapenemase-producers carriage. Results of rapid diagnostic workflow were compared with those obtained by conventional diagnostic routine.

Results: Overall, the direct MALDI-TOF MS protocol allowed reliable identification to the species level of 92.1% of the 2133 monomicrobial BCs. Rate of matched identification was significantly higher for Enterobacterales (97.3%) in comparison to non-fermenting Gram-negative species (80.2%), obligate anaerobic bacteria (42.1%) and fastidious Gram-negative species (41.5%). The overall sensitivity of NG-Test® CARBA 5 and NG-Test® CTX-M MULTI was 92.2% and 91.6%, respectively. Integration of Easyplex® SuperBug CRE allowed the detection of blaKPC mutants associated with ceftazidime/avibactam resistance, reaching 100% sensitivity in carbapenemase detection. Both LFIAs and molecular testing showed no false-positive results.

Conclusions: Algorithms based on MALDI-TOF MS, LFIAs and molecular testing may represent a cost-effective tool to timely identify Gram-negative species and detect resistance markers directly from BCs. According to local epidemiology, these results may allow antimicrobial stewardship interventions including prompt use of new approved drugs.