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Evaluation of The Ng-Test Carba 5 for Rapid Detection of Carbapenemases in Clinical Isolates of *Klebsiella Pneumoniae*

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Abstract

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKp) is a critical global health threat due to its multidrug resistance, primarily driven by carbapenemase production. Rapid and accurate detection of carbapenemases is essential for effective treatment and infection control. This study evaluates the validity of the NG-Test Carba 5, a rapid immunochromatographic assay, for detecting five major carbapenemases (KPC, NDM, VIM, IMP, OXA-48-like) in clinical CRKp isolates. **Methods:** Clinical isolates of CRKp were collected from various clinical specimens at the Military Medical Academy in Belgrade, Serbia, between January 2023 and October 2024. Detection of carbapenemases was performed using NG-Test Carba 5, while PCR served as the reference method. Diagnostic performance was assessed by calculating sensitivity, specificity, and Cohen's kappa coefficient**.** **Results:** Among 312 isolates, OXA-48-like was the most prevalent carbapenemase. NG-Test Carba 5 showed high sensitivity (98.7%) and specificity (100%) overall, with excellent agreement for NDM (κ = 0.947), OXA-48-like (κ = 0.957), and KPC (κ = 0.978). However, it failed to detect VIM in five PCR-positive isolates, suggesting potential limitations. **Conclusion:** NG-Test Carba 5 is a rapid and reliable tool for detecting major carbapenemases in CRKp, though its performance for VIM detection requires further investigation. This assay has the potential to improve clinical diagnostics and strengthen infection control in settings with high antimicrobial resistance.

Keywords: *Klebsiella pneumoniae*; carbapenemase; NG-Test Carba 5; PCR; antimicrobial resistance; rapid diagnostics

1. Introduction

Antimicrobial resistance a major global health threats of the 21st century. Due to its ability to rapidly acquire resistance, *Klebsiella pneumoniae* (*K. pneumoniae*) has been classified by the World Health Organization (WHO) as a critical priority pathogen [1]. The underlying cause is the interplay between diverse virulence determinants and the broad acquisition of antimicrobial resistance mechanisms among *K. pneumoniae* isolates [2]. The most common of these mechanism is the production of carbapenemases [3]. As the main source of resistance in carbapenem-resistant *Klebsiella. pneumoniae* (CRKp), carbapenemases are divided into three Ambler classes based on their amino acid sequences: Ambler class A (K. pneumoniae carbapenemase, (KPC), Ambler class B (metallo-β-lactamases (MBL), including New Delhi metallo-β-lactamase, (NDM); Verona integron-encoded metallo-β-lactamase, (VIM), and imipenemase (IMP)), and Ambler class D (oxacillinase β-lactamase-48, (OXA-48-like) carbapenemase) [4].

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Carbapenemase-producing *Klebsiella pneumoniae* (CPKp) can lead to life-threatening infections [5,6]. Accurate and timely detection of carbapenemase production is essential in routine microbiological diagnostics, as it directly influences both clinical decision-making and infection control strategies [5–7]. Additionally, because the susceptibility of isolates containing different carbapenemases may differ in order to properly direct antibiotic therapy, it is important to accurately identify serine versus MBL carbapenemases. For example, the β-lactam/β-lactamase inhibitor ceftazidime/avibactam combination works well against class A and class D carbapenemases, but not against class B metalloenzymes [8], which have the ability to hydrolyze the new β-lactamase inhibitors [9]. The NG-Test Carba 5 (NG Biotech, Guipry, France) is a immunochromatographic assay that utilizes monoclonal antibodies to detect the five major carbapenemases (KPC, NDM, VIM, IMP, and OXA-48-like) directly from bacterial colonies, offering results within 15 minutes through a simple procedure and easy-to-interpret test strips [10–13].

Given the limited number of studies evaluating the performance of the NG-Test Carba 5 under real laboratory conditions, particularly those testing a variety of real and mixed clinical specimens (combining both real clinical and spiked cultures), rather than artificially inoculated media (5, 12, 14, 15, 16), our study aimed to evaluate the perfomance and clinical usefulness of the NG-Test Carba 5, for routine laboratory use, in detecting and distinguishing the five major carbapenemases among clinical isolates of CPKp. Conventional PCR was used as the reference standard to evaluate the test accuracy within a typical diagnostic workflow.

2. Methods

2.1. Isolates

Between January 2023 to October 2024, 312 strains of CRKp isolated from clinical samples originating from different wards and various clinical specimens in the Military Medical Academy, Serbia, were included in the study. All samples were cultured on blood agar with 5% sheep blood (bioMérieux, France) and incubated overnight at 37°C in an aerobic atmosphere. After incubation, colonies consistent with *Klebsiella* morphology were identified to the species level by the MALDI-TOF MS method (Vitek MS, bioMérieux, France).

Susceptibility to carbapenem antibiotics was performed during the routine antibiotic testing diagnostic procedure by using the disc diffusion method according to Kirby-Bauer [17]. Resistance to carbapenem antibiotics was confirmed with meropenem (10μg) and ertapenem (10μg) discs (BioRad, France) according to the EUCAST methodology. For some of species, the minimal inhibitory concentration (MIC) of meropenem and ertapenem was also determined by the gradient test method ETEST (Liofilhem, Italy).

According to EUCAST guidelines, *K. pneumoniae* isolates that demonstrated an inhibition zone for ertapenem or/and meropenem <25 mm using the disc diffusion method and < 2 mg/L by gradient test method were chosen for additional examination [18].

2.2. NG-Test Carba 5 Assay

The NG-Test Carba 5 Assay, for the five major carbapenemases KPC, IMP, NDM, VIM, and OXA-48-like, was carried out in accordance with the manufacturer's instructions (NG Biotech, Guipry, France). In short, 100 μL of the mixture was poured onto the CARBA-5 cassette after a 1 μL loopful of bacteria and five drops of extraction buffer were combined. The mixture should be vortexed and then allowed to sit at room temperature (20 to 25 degrees) for 15 minutes. After 15 minutes of room temperature incubation, the results were evaluated [13].

2.3. DNA Isolation and Detection of Carbapenemase Genes by PCR

DNA was extracted using the boiling method. A couple of colonies from an agar plate were transferred to Luria-Bertani Broth (LB) medium and incubated overnight. From the overnight culture,

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1500 µl of bacteria were centrifuged for 2 minutes at 12000 rpm. The pellet was resuspended in 300 µl of sterile, distilled water, boiled for 10 minutes, and transferred to -20ºC for 10 minutes. After that, tubes were centrifuged for 2 minutes at 12000 rpm, and the supernatant containing the DNA was transferred to a new tube and used for PCR or kept at -20ºC. Detection of carbapenemase genes was carried out using primers in Table 1. The genes *bla*NDM, *bla*KP, *bla*OXA-48, and *bla*VIM were detected by multiplex PCR reaction. The PCR conditions were initial denaturation at 94 ºC for 5 min, followed by 30 cycles of denaturation a 94 ºC for 45 s, annealing at 59 ºC for 60 s, and extension at 72 ºC for 60 s, and the final extension at 72 ºC for 10 min. The PCR prducts were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized using a UV transilluminator [20–23].

Table 1. Primers used for carbapenemases genes detection.

Primer	Sequence 5′-3′	Amplicon size (bp)	Reference
*bla*KPC Fw	ATGTCACTGTATCGCCGTCT	893	[20]
*bla*KPC Rw	TTTTCAGAGCCTTACTGCCC		
*bla*VIM Fw	GATGGTGTTTGGTCGCATA	390	[21]
*bla*VIM Rw	CGAATGCGCAGCACCAG		
*bla*NDM Fw	GGGCAGTCGCTTCCAACGGT	475	[22]
*bla*NDM Rw	GTAGTGCTCAGTGTCGGCAT		
*bla*IMP Fw	GGAATAGAGTGGCTTAATTCTC	188	[21]
*bla*IMP Rw	CCAAACCACTACGTTATCT		
*bla*OXA-48-like Fw	TTGGTGGCATCGATTATCGG	744	[23]
*bla*OXA-48-like Rw	GAGCACTTCTTTTGTGATGGC		

KPC – *Klebsiella pneumoniae* carbapenemase; VIM – Verona integron-encoded metallo--lactamase; NDM – New Delhi metallo-β-lactamase; IMP – imipenemase; OXA-48-like – Oxacillinase-48-like.

2.4. Statistical Analysis

The NG-test Carba 5 sensitivity and specificity were computed using a 95% confidence interval (CI). Each method's agreement with the PCR as gold standard was assessed using the Kappa index (κ index). SPSS Statistics v15.0 was used to analyze the data (IBM, Chicago, IL, USA).

3. Results

A total of 312 isolates were analyzed. Blood cultures were the most frequent specimen source (54.8%), followed by aspirates (21.5%) and urine cultures (10.9%). Confirmatory PCR analysis revealed that the most commonly detected gene was *bla*OXA-48-like (67.3%), followed by *bla*NDM (24.7%) and *bla*KPC (18.3%). *bla*VIM was detected in 1.6% of isolates, while *bla*IMP was not detected. Correspondingly, the NG-Test Carba 5 identified OXA-48-like in 65.4% and NDM in 22.8% of samples, with no detection of VIM or IMP. The summarized concordance in gene detection demonstrated that the majority of isolates harbored a single gene (87.2% by PCR; 90.4% by NG-Test Carba 5), whereas dual gene presence was observed in a smaller proportion (11.9% by PCR; 7.7% by NG-Test Carba 5). PCR identified the simultaneous presence of three genes in only one isolate (0.3%) (Table 2). Comparison of the diagnostic performance of the NG-Test Carba 5 with PCR as the reference method for detecting individual carbapenemase genes showed that the NG-Test Carba 5 demonstrated high sensitivity and perfect specificity for NDM (92.2% sensitivity, 100% specificity), OXA-48-like (97.1%, 100%), and KPC (96.5%, 100%) (Table 2). For VIM and IMP, NG-Test Carba 5 showed no positive results, resulting in undefined sensitivity due to the absence of positive PCR-confirmed cases for *bla*IMP, and low detection for *bla*VIM. The overall sensitivity and specificity of NG-Test Carba 5 in comparison to PCR for all genes combined were 98.7% and 100%, respectively. The positive predictive value was 100% for all targets, while the negative predictive value ranged from 94.4% to 99.2%, except for the total PCR-negative cases, where it was 66.7% (Table 3). The Table 4 summarizes the agreement between NG-Test Carba 5 and PCR results using Cohen's kappa

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coefficient. There was excellent agreement for the detection of NDM (κ = 0.947), OXA-48-like (κ = 0.957), and KPC (κ = 0.978), all with statistically significant p-values (<0.001). No agreement was observed for VIM (κ = 0.000, p = 1.000), and kappa values for IMP could not be calculated due to the absence of positive cases. The overall agreement between NG-Test Carba 5 and PCR across all gene targets was moderate (κ = 0.495, p < 0.001) (Table 3).

Table 2. Characteristics of Sample Isolates with PCR-Identified Genes and Enzymes Detected by NG-Test Carba 5.

Categories		Frequency	Percent (%)
Specimen	Urine culture	34	10.9
	Blood culture	171	54.8
	BALL	13	4.2
	Aspirate	67	21.5
	Kiss swab	25	8.0
	Liquor	2	0.6
PCR-NDM	Negative	235	75.3
	Positive	77	24.7
PCR-OXA48	Negative	102	32.7
	Positive	210	67.3
PCR-KPC	Negative	255	81.7
	Positive	57	18.3
PCR-VIM	Negative	307	98.4
	Positive	5	1.6
PCR-IMP	Negative	312	100.0
	Positive	0	0.0
NG-Test Carba5/NDM	Negative	241	77.2
	Positive	71	22.8
NG-Test Carba5/OXA48	Negative	108	34.6
	Positive	204	65.4
NG-Test Carba5/KPC	Negative	257	82.4
	Positive	55	17.6
NG-Test Carba5/VIM	Negative	312	100.0
	Positive	0	0.0
NG-Test Carba5/IMP	Negative	312	100.0
	Positive	0	0.0
All PCR	Negative	2	0.6
	One gene detected	272	87.2
	Two genes detected	37	11.9
	Three genes detected	1	0.3
All NG-Test Carba5	Negative	6	1.9
	Single carbapenemase detected	282	90.4
	Double carbapenemases detected	24	7.7

Table 3. Diagnostic Performance of NG-Test Carba 5 Compared to PCR as the Reference Method for Carbapenemase Gene Detection.

Results by reference methods (n)	NG Carba 5							
	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PCR-NDM	71	0	235	6	92.2	100.0	100.0	97.5
PCR-OXA48	204	0	102	6	97.1	100.0	100.0	94.4

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PCR-KPC	55	0	255	2	96.5	100.0	100.0	99.2
PCR-VIM	0	0	307	5	/	100.0	/	98.4
PCR-IMP	0	0	312	0	/	100.0	/	100.0
ALL PCR	306	0	2	4	98.7	100.0	100.0	66.7

Table 4. Agreement Between NG-Test Carba 5 and PCR Results for Detection of Carbapenemase (Cohen's Kappa Analysis).

PCR vs. NG-Test Carba 5	Kappa	*p*
PCR-*bla*$_{NDM}$ vs. NG Carba 5 NDM	0.947	<0.001
PCR-*bla*$_{OXA48\text{-}like}$ vs. NG Carba 5 OXA48-like	0.957	<0.001
PCR-*bla*$_{KPC}$ vs. NG Carba 5 KPC	0.978	<0.001
PCR-*bla*$_{VIM}$ vs. NG Carba 5 VIM	0.000	1.000
PCR-*bla*I$_{MP}$ vs. NG Carba 5 IMP	/	/
ALL *bla*PCR vs. all NG Carba 5	0.495	<0.001

4. Discussion

The emergence and spread of CRKp represent a significant public health concern due to the rapid development of resistance especially in hospital settings. Carbapenem resistance, primarily mediated by the production of carbapenemases, highlights the urgent need for rapid diagnostics and appropriate therapeutic strategies. (24,25) Many studies have indicated Carbapenemase-producing Enterobacteriaceae (CPE) as the predominant type of Carbapenem-resistant Enterobacteriaceae (CRE,) with prevalence rates ranging from 77.3% to 91.3% [26–28]. Also, in our study, 99.36% (310/312) of clinical CRKp produced carbapenemases, indicating that the main mechanism of resistance among clinical CRKp isolates is carbapenemase production. Of the 310 CPKp isolates, OXA-48-like carbapenemase was the most prevalent, which is consistent with a study of *K. pneumoniae* in community settings in Belgrade, Serbia [29]. CPKp is often resistant to multiple antibiotics and has limited treatment options. In terms of treatment, the choice of drugs to treat CRE infection depends on specific carbapenemases [30]. For KPC and OXA-48-like enzymes, ceftazidime-avibactam can be the preferred agent [31]. Unfortunately, ceftazidime-avibactam is not active on MBL producers, and other options must be investigated, such as combining the remaining active antibiotics. (e.g., colistin, tigecycline, aminoglycosides, and/or fosfomycin) [32], or aztreonam in combination with ceftazidime-avibactam. (33,34). Consequently, it is crucial to detect and identify carbapenemases as soon as possible, so that the physician can quickly apply or change antibiotic therapy and implement appropriate infection control measures. NG-Test Carba 5 is an effective, quick, and practical diagnostic technique that may aid in streamlining the intricate regular workflow for carbapenemase detection.

In our study, by comparing NG-Test Carba 5 to the reference PCR method, the overall sensitivity and specificity were 98.7% and 100%, respectively. Our results show excellent performance of NG-Test CARBA 5 for detecting major variants of carbapenemases (OXA-48-like and KPC) among CPKp (sensitivity and specificity >95%). However, it is important to note that the NG-Test Carba 5 gave a false negative result for four CPKp isolates and missed four single NDM-producing strains. Similar to ours are the results obtained by Baer *et al*. who tested isolates for the presence of all five types of carbapenemase. They correctly identified 89.5% of the strains tested, while 10.5% of the isolates showed a false negative result. They identified 80% of NDM, 83.3% of VIM, 87.5% of OXA-48-like, and 100% of KPC producers. Authors also observed that the test failed to detect a single IMP-producing pathogen [35]. Contrary to our study, Pruss *at al* detected 100% of strains producing carbapenemases of the NDM, VIM, KPC, and OXA-48-like types, and negative results were obtained for strains not producing carbapenemases [36].

In addition to the four NDM isolates passed, the other missed carbapenemases in our study are from isolates coproduceding two or three types of carbapenemases, including NDM+VIM (n=2),

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OXA-48 like +VIM, (n=2), KPC+VIM (n=1), NDM + OXA-48-like like (n=5), OXA+KPC (n=4) and NDM+OXA+KPC (n=1). In contrast, twenty-four coproducing isolates (NDM+ OXA-48-like (n=24) were accurately identified by NG-Test Carba 5, suggesting that strains producing multiple carbapenemases are very common in our hospital environment. Also, Liu *et al*. showed that the NG test missed 8 NDM, 4 OXA-48-like, and 1 IMP blood isolates, especially when co-carriage of carbapenemases compared with the Xpert Carba-R assay [37]. On the other hand, in a study from Guangdong, China, seven isolates coproduced two types of carbapenemases, including NDM+IMP (n=5), KPC+NDM (n=1), and OXA-48-like+IMP, n=1), were accurately identified by NG – Test CARBA 5 [38].

Most importantly, in our study, the accuracy of the NG – Test CARBA 5 may also be affected by variants of carbapenemases, especially false-negative results for detecting VIM. Five CPKp isolates harboring the *bla*VIM gene were detected by PCR analysis but were missed by the NG-Test Carba 5 assay. There are several possible explanations for this. Firstly we speculated on possibility that bacterial colonies utilized to prepare immunochromatographic tests and DNA isolation may contain clones that were not recognized, and which could influence our data analysis. Secondly, there may be a false negative result due to the nature of the NG-Test Carba 5 method (a multiplex immunochromatographic assay, which is based on the detection of expressed proteins by antibodies) if the carbapenemases are not well expressed or if contain amino acid substitutions at the primary epitope of the antibody [39]. Finally, a PCR may yield false-positive results if a mutation alters the carbapenemase activity but not the primer binding sites or gene length [40].

Notable limitation of our study concerns the detection of the VIM-producing *K. pneumoniae* isolates. While the NG-Test Carba 5 assay did not detect VIM carbapenemase, PCR analysis identified five *K. pneumoniae* isolates carrying the *bla*VIM gene. Since we were unable to perform gene sequencing analysis to confirm PCR findings, the possibility of false positive results, non-specific amplification, or the presence of a non-functional or partial gene sequence cannot be ruled out. Thus, we cannot state with certainty whether the NG-Test Carba 5 failed to detect VIM-positive isolates. In order to fully assess the sensitivity and specificity of the NG-Test Carba 5 in detecting VIM carbapenemase, further studies that incorporate sequencing analysis are necessary to definitively confirm the presence of the *bla*VIM gene and validate the observed discrepancy between the PCR and NG-Test Carba 5 results. **Saito et al.** also highlighted in their study that the detection of **VIM carbapenemases**, particularly those with low expression levels, remains a challenge for **NG-Test Carba 5 [16].**

Our study confirms that NG-Test CARBA 5 is highly accurate in detecting the most common carbapenemases among clinical CPKp isolates, with an overall sensitivity of 98.7% and specificity of 100% when compared to the PCR method. These results largely align with studies that used real clinical samples, such as the research by Boutal et al., which demonstrated a high sensitivity of 97.7% and specificity of 96.1% in clinical blood cultures [12]. Additionally, our findings are consistent with studies that used a mixed culture, combining real clinical and spiked cultures, where sensitivity ranged from 94-98% and specificity from 97-99% for NG-Test CARBA 5 (5, 14, 15,16). **Qin et al.**, in their systematic review of various studies evaluating the validity of the **NG-Test Carba 5**, highlighted the importance of using **real clinical samples** in diagnostic assessments. Their review indicated that while **spiked cultures** are valuable for controlled testing, they do not fully replicate the complexities of **patient-derived isolates**. These complexities, such as mixed bacterial populations, host factors, and microbial interactions, can significantly influence the performance and accuracy of diagnostic tests like NG-Test Carba 5. The review also noted a **difference in sensitivity and specificity** between real clinical samples and spiked cultures. Specifically, studies using **real clinical samples** often reported higher **sensitivity** and **specificity** compared to those using **spiked cultures**, where test performance could be affected by the artificial nature of the samples [41]. Therefore, our data highlight the advantages of using real clinical samples in the evaluation of diagnostic tests. Our study strongly supports the use of real clinical samples in assessing NG-Test CARBA 5, as it provides a more realistic view of its diagnostic accuracy and reliability.

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5. Conclusions

Based on our results and those of previous studies, it can be concluded that NG-Test CARBA 5 is a rapid, reliable, and easy to implement in routine workflow tool for detecting major carbapenemases in CRKp, The assay could be very useful in assistance with clinical diagnostics, optimal therapy choice as well as infection control in high-resistance clinical settings. It showed high accuracy with good sensitivity and specificity for detection of the clinically significant carbapenemases NDM, OXA-48-like, and KPC, though its performance for VIM detection requires further investigation.

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