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Evaluation of an immunochromatographic method (NG-TEST® CARBA-5) for detection of different allelic variants of KPC, NDM, IMP and VIM carbapenemases in Enterobacterales (ETB) and *Pseudomonas aeruginosa* (PAE) isolates

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BACKGROUND
Accurate detection of carbapenemase (CBP)-mediated resistance is crucial for guide antibiotic therapy and effective infection control measures. NG-Test CARBA-5 (NG-Biotech) is a rapid, multiplex, lateral immunochromatographic assay designed to identify the five most common CBP: KPC/OXA-48-like/VIM/IMP/NDM, within 15 minutes from bacterial colonies in ETB and PAE.

OBJETIVE
This study aimed to verify the diagnostic performance for detecting a diversity of KPC, NDM, IMP and VIM allelic variants in isolates of ETB and PAE.

MATERIALS AND METHODS
We evaluated a collection of 74 strains from the repository of INEI-ANLIS-Malbrán, CDC-AR-BANK, and NDM constructs obtained by targeted mutations and cloned in *E. coli*-ECO- DH5α/pMBL at IBR-CONICET. CBP alleles were characterized by PCR+DNA-sequencing and/or WGS. The collection included: 47 isolates of ECO (2 clinical isolates with KPC-4 and NDM-1, and 45 vector isolates with either NDM-1 to NDM-31, NDM-33 to NDM-42, NDM-44 to NDM-46, and NDM-16b-substitution V88L+M154L+A233V), 12 PAE (IMP-1, IMP-14, IMP-15, IMP-16, IMP-18, IMP-26, 2 VIM-2, VIM-4, VIM-11, KPC-2, KPC-5), 9 *K. pneumoniae*-KPN- (IMP-4, IMP-8, VIM-1, VIM-27, KPC-2, KPC-3, KPC-14, NDM-1, NDM-5), 2 *E. cloacae* (IMP-13, KPC-3), 2 *P. rettgeri*-PRE- (IMP-27, IMP-67), 1 *K. aerogenes* (IMP-4) and 1 *M. morganii* (NDM-1). NG-Test CARBA-5 underwent evaluation in accordance with the manufacturer's instructions. ECO vectors were cultured with 100µM IPTG. False negative results were retested with 15-minute incubation (room temperature) of the bacterial suspension with extraction buffer. Acceptability criteria: sensitivity (SE), specificity (SP) and precision (PR) >= 95%.

RESULTS
NG-Test CARBA-5 detected all variants of NDM and VIM and most of KPC (6/7) and IMP (9/12). False negative results were *Klebsiella pneumoniae* KPC-14, *Providencia rettgeri* IMP-27 and IMP-67 and *Pseudomonas aeruginosa* IMP-16. The assay demonstrated 95% SE, 100% SP and 99% PR. False-negative results (n=4) persisted upon retesting with an extended extraction period.

CONCLUSIONS
NG-Test CARBA-5 exhibited a high-level of performance, with >=95% of SE/SP/PR. These parameters could potentially be influenced by locally prevalent allelic variants. However, the assay effectively detects the most globally widespread variants, offering a promising outcome and a significant advancement for routine diagnosis.

Collection of strains evaluated with NG-TEST®CARBA-5

INEI-ANLIS ID	AR BANK ID	ORGANISM	RESISTANCE MECHANISM
M27846	103	*Pseudomonas aeruginosa*	IMP-1, OXA-50, PAO
M27848	34	*Klebsiella pneumoniae*	IMP-4, TEM-1B, SHV-11
M27847	161	*Enterobacter aerogenes*	IMP-4, TEM-1B, SFO-1, OXA-1
M28573	-	*Klebsiella pneumoniae*	IMP-8
M27863	1110	*Enterobacter cloacae*	IMP-13
M27864	92	*Pseudomonas aeruginosa*	IMP-14
M27865	1114	*Pseudomonas aeruginosa*	IMP-15
M11041	-	*Pseudomonas aeruginosa*	IMP-16
M27866	1112	*Pseudomonas aeruginosa*	IMP-18
M27868	1116	*Pseudomonas aeruginosa*	IMP-26
M27869	1113	*Providencia rettgeri*	IMP-27
M27871	1111	*Providencia rettgeri*	IMP-67
M27842	76	*Klebsiella pneumoniae*	VIM-1, SHV-30
M27843	100	*Pseudomonas aeruginosa*	VIM-2, OXA-50, PAO
M27860	108	*Pseudomonas aeruginosa*	VIM-2, OXA-50, PAO, OXA-4
M27845	54	*Pseudomonas aeruginosa*	VIM-4, OXA-50, PAO
M5109	-	*Pseudomonas aeruginosa*	VIM-11
M27844	46	*Klebsiella pneumoniae*	VIM-27, CTX-M-15, SHV-11, OXA-1
M27829	356	*Pseudomonas aeruginosa*	KPC-2
BAA1705	-	*Klebsiella pneumoniae*	KPC-2
M27831	2	*Enterobacter cloacae*	KPC-3, OXA-9, TEM-1A
M27832	347	*Klebsiella pneumoniae*	KPC-3
M27833	104	*Escherichia coli*	KPC-4, TEM-1A
M27855	90	*Pseudomonas aeruginosa*	KPC-5
M27054	-	*Klebsiella pneumoniae*	KPC-14
M27649	-	*Klebsiella pneumoniae*	NDM-1, CTX-M, CMY
M27836	57	*Morganella morganii*	NDM-1, CTX-M-15, OXA-1
M27837	69	*Escherichia coli*	NDM-1, TEM-1B, CMY-6
M28206	-	*Klebsiella pneumoniae*	NDM-5, CTX-M
E.coli DHS α/pMBL-ST-1	-	*Escherichia coli*	NDM-1
E.coli DHS α/pMBL-ST-2	-	*Escherichia coli*	NDM-2
E.coli DHS α/pMBL-ST-3	-	*Escherichia coli*	NDM-3
E.coli DHS α/pMBL-ST-4	-	*Escherichia coli*	NDM-4
E.coli DHS α/pMBL-ST-5	-	*Escherichia coli*	NDM-5
E.coli DHS α/pMBL-ST-6	-	*Escherichia coli*	NDM-6
E.coli DHS α/pMBL-ST-7	-	*Escherichia coli*	NDM-7
E.coli DHS α/pMBL-ST-8	-	*Escherichia coli*	NDM-8
E.coli DHS α/pMBL-ST-9	-	*Escherichia coli*	NDM-9
E.coli DHS α/pMBL-ST-10	-	*Escherichia coli*	NDM-10
E.coli DHS α/pMBL-ST-11	-	*Escherichia coli*	NDM-11
E.coli DHS α/pMBL-ST-12	-	*Escherichia coli*	NDM-12
E.coli DHS α/pMBL-ST-13	-	*Escherichia coli*	NDM-13
E.coli DHS α/pMBL-ST-14	-	*Escherichia coli*	NDM-14
E.coli DHS α/pMBL-ST-15	-	*Escherichia coli*	NDM-15
E.coli DHS α/pMBL-ST-16	-	*Escherichia coli*	NDM-16
E.coli DHS α/pMBL-ST-16.b	-	*Escherichia coli*	NDM-16b
E.coli DHS α/pMBL-ST-17	-	*Escherichia coli*	NDM-17
E.coli DHS α/pMBL-ST-18	-	*Escherichia coli*	NDM-18
E.coli DHS α/pMBL-ST-19	-	*Escherichia coli*	NDM-19
E.coli DHS α/pMBL-ST-20	-	*Escherichia coli*	NDM-20
E.coli DHS α/pMBL-ST-21	-	*Escherichia coli*	NDM-21
E.coli DHS α/pMBL-ST-22	-	*Escherichia coli*	NDM-22
E.coli DHS α/pMBL-ST-23	-	*Escherichia coli*	NDM-23
E.coli DHS α/pMBL-ST-24	-	*Escherichia coli*	NDM-24
E.coli DHS α/pMBL-ST-25	-	*Escherichia coli*	NDM-25
E.coli DHS α/pMBL-ST-26	-	*Escherichia coli*	NDM-26
E.coli DHS α/pMBL-ST-27	-	*Escherichia coli*	NDM-27
E.coli DHS α/pMBL-ST-28	-	*Escherichia coli*	NDM-28
E.coli DHS α/pMBL-ST-29	-	*Escherichia coli*	NDM-29
E.coli DHS α/pMBL-ST-30	-	*Escherichia coli*	NDM-30
E.coli DHS α/pMBL-ST-31	-	*Escherichia coli*	NDM-31
E.coli DHS α/pMBL-ST-33	-	*Escherichia coli*	NDM-33
E.coli DHS α/pMBL-ST-34	-	*Escherichia coli*	NDM-34
E.coli DHS α/pMBL-ST-35	-	*Escherichia coli*	NDM-35
E.coli DHS α/pMBL-ST-36	-	*Escherichia coli*	NDM-36
E.coli DHS α/pMBL-ST-37	-	*Escherichia coli*	NDM-37
E.coli DHS α/pMBL-ST-38	-	*Escherichia coli*	NDM-38
E.coli DHS α/pMBL-ST-39	-	*Escherichia coli*	NDM-39
E.coli DHS α/pMBL-ST-40	-	*Escherichia coli*	NDM-40
E.coli DHS α/pMBL-ST-41	-	*Escherichia coli*	NDM-41
E.coli DHS α/pMBL-ST-42	-	*Escherichia coli*	NDM-42
E.coli DHS α/pMBL-ST-44	-	*Escherichia coli*	NDM-44
E.coli DHS α/pMBL-ST-45	-	*Escherichia coli*	NDM-45
E.coli DHS α/pMBL-ST-46	-	*Escherichia coli*	NDM-46