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Analytical Concordance Between The Modified Hodge Test, Carba NP, And Immunochromatographic Lateral Flow Assays For KPC And NDM Detection In Clinical Enterobacterales: A Systematic Review And Meta-Analysis Of Bench Validation Studies

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Abstract

Background: Rapid identification of KPC and NDM carbapenemases in Enterobacterales is essential for targeted therapy and infection prevention. Objective: To evaluate and compare the analytical performance and concordance of three assays—Modified Hodge Test (MHT), Carba NP, and lateral-flow immunochromatographic assays (LFIAs)—against molecular reference standards. Methods: We conducted a PRISMA-2020–compliant systematic review of PubMed, Embase, Scopus, Web of Science, and Cochrane CENTRAL (January 2010–October 2025) with PROSPERO registration. Eligible studies were bench or diagnostic-accuracy evaluations of clinical Enterobacterales with PCR/sequencing confirmation.

Two reviewers screened, extracted 2×2 data, and assessed quality using QUADAS-2. Accuracy was summarized with bivariate random-effects and HSROC models; analyses examined heterogeneity, subgroup effects (enzyme, species, setting, matrix), meta-regression, and Deeks' test for small-study effects. Results: Across evaluations, LFIAs achieved the highest and most consistent accuracy for KPC and NDM with rapid turnaround and excellent concordance with PCR. Carba NP provided high specificity and good sensitivity under optimized buffers, inoculum, and readout but showed protocol-dependent variability.

MHT yielded acceptable KPC detection in enriched panels yet demonstrated poor specificity for NDM and substantial heterogeneity, consistent with its archival status in standards. Sensitivity analyses and head-to-head comparisons supported the hierarchy LFIA ≥ optimized Carba NP ≫ MHT. Conclusions: LFIAs should be prioritized as the frontline test for KPC/NDM typing, with standardized Carba NP as an alternative where needed and molecular confirmation for discrepant or high-consequence results. Retiring MHT from screening workflows and adopting LFIA-anchored algorithms can shorten time to

targeted therapy, strengthen infection-prevention measures, and improve surveillance fidelity across resource settings.

Introduction

Carbapenem-resistant Enterobacterales (CRE) have established themselves as a high-impact threat in both acute-care and community settings, driven by rapid, plasmid-mediated dissemination of carbapenemase genes among Klebsiella pneumoniae, Escherichia coli, and related species (CDC, 2024). Global surveillance coordinated through WHO's GLASS shows persistently high and, in many regions, rising resistance to last-line agents, with substantial gaps in reporting from low- and middle-income countries that likely understate the true burden (WHO, 2025). European data synthesized by ECDC indicate sustained transmission pressure, increasing bloodstream infection (BSI) incidence in many EU/EEA member states, and explicit regional targets to reduce carbapenem-resistant K. pneumoniae BSIs by 2030, underscoring the continuing public-health urgency (ECDC, 2025). In parallel, multicountry clinical cohorts and meta-analyses consistently associate CRE infection with excess mortality, prolonged hospitalization, and resource utilization compared with infections caused by carbapenem-susceptible Enterobacterales (Baek et al., 2024).

Geographically, the distribution of specific carbapenemases is heterogeneous. KPC enzymes—Ambler class A serine carbapenemases—predominate across the Americas and parts of Europe, whereas metallo-β-lactamases (MBLs) such as NDM—Ambler class B—are highly prevalent in South Asia and increasingly reported worldwide (ECDC, 2025; Park, 2024). This evolving landscape is complicated by travel, medical tourism, and the horizontal transfer of mobile genetic elements, which facilitate the rapid inter-species spread of carbapenemase genes and the emergence of co-producers (e.g., KPC+NDM) with narrower therapeutic options and worse outcomes (CDC, 2024; Li et al., 2024). Together, these trends sustain a cycle in which empiric therapy fails more often, stewardship becomes more complex, and infection-prevention programs face escalating demands for rapid and accurate detection to contain outbreaks. Against this backdrop, laboratories require diagnostic methods that are both analytically reliable and operationally feasible across diverse resource settings, enabling timely clinical and epidemiological decisions. (Citations: ECDC, 2025; WHO/GLASS, 2025; CDC, 2024; Baek et al., 2024; Park, 2024; Li et al., 2024.)

Clinical importance of KPC and NDM carbapenemases

KPC and NDM are the most consequential carbapenemases in clinical Enterobacterales because they erode the efficacy of carbapenems and many β-lactam/β-lactamase inhibitor (BL/BLI) combinations. KPC enzymes are generally inhibited by avibactam, rendering ceftazidime-avibactam (CZA) a cornerstone for KPC-producing infections when susceptibility is confirmed (IDSA, 2024; Kang et al., 2024). By contrast, NDM enzymes are zinc-dependent MBLs not inhibited by avibactam; therapeutic strategies often rely on the aztreonam-avibactam (ATM-AVI) combination (or aztreonam plus CZA in staged or co-administration regimens) to exploit aztreonam's MBL stability while counteracting co-produced serine β-lactamases (Sader et al., 2025; BMCID, 2024). Clinical series and observational cohorts link CRE BSIs—especially those due to KPC and NDM producers—to high 30-day mortality, with co-production (e.g., KPC+NDM) conferring particularly poor prognoses (Li et al., 2024; Baek et al., 2024).

From an infection-control perspective, KPC and NDM are sentinel markers of transmission risk because they are commonly plasmid-encoded, readily transferable, and increasingly detected outside traditional hotspots. Their timely detection informs isolation decisions, contact tracing, and targeted decolonization or device management. Consequently, accurate, rapid, and scalable laboratory algorithms that can discriminate KPC and NDM—and do so close to the bench—are vital for optimizing therapy and preventing onward spread. (Citations: IDSA, 2024; Kang et al., 2024; Sader et al., 2025; BMCID, 2024; Li et al., 2024; Baek et al., 2024.)

Overview of diagnostic tools

Modified Hodge Test (MHT). The MHT detects carbapenemase activity indirectly via the "cloverleaf" enhancement of indicator-strain growth around a carbapenem disk. Although simple and inexpensive, MHT suffers from poor specificity—particularly false positives with ESBL or AmpC producers plus porin loss—and variable performance against MBLs such as NDM. Reflecting these limitations, CLSI archived MHT after 2017 and now deems it unreliable compared with newer methods (e.g., mCIM, Carba NP) (CLSI, 2024; Pasteran et al., 2010; CMR, 2024). In laboratories that still employ MHT for preliminary screening due to cost constraints, confirmatory testing with more specific assays remains essential.

Carba NP. Carba NP is a rapid biochemical assay that detects in vitro hydrolysis of imipenem through a pH-indicator color shift, typically within 30–120 minutes (Nordmann, Poirel, & Dortet, 2012). Multicenter evaluations report high overall accuracy and broad applicability across Enterobacterales and non-fermenters, though performance can fluctuate with inoculum, buffer composition, and enzyme type (e.g., OXA-48-like) (Cunningham et al., 2017; Poirel et al., 2015). Numerous modified protocols (e.g., MCNP) aim to improve sensitivity for difficult enzyme families or streamline workflow in routine labs. Carba NP's low equipment requirements and speed make it attractive for bench validation, but lot-to-lot variability and operator technique can introduce heterogeneity across studies.

Lateral-flow immunochromatographic assays (LFIA). LFIA kits (e.g., NG-Test CARBA 5; RESIST-5 O.O.K.N.V./O.K.N.V.I.) use monoclonal antibodies to capture carbapenemase proteins directly, providing genotype-level results (KPC, NDM, VIM, IMP, OXA-48-like) in ~10–15 minutes from colonies or positive blood-culture pellets. Bench and clinical evaluations consistently show very high sensitivity and specificity for KPC and NDM, with strong agreement versus PCR (Han et al., 2021; Lauwerier et al., 2024; Qin et al., 2025). Recent multi-center data—including 2024–2025 assessments—corroborate robust performance across diverse matrices and co-producers, while noting rare cross-reactivity events and the inherent limitation that only targeted enzymes are detected (Calica et al., 2025; Munguía-Ramos et al., 2024). Operationally, LFIAs minimize hands-on time, require no specialized instrumentation, and are well suited to outbreak investigations and rapid bench validation pipelines.

3.2 Rationale

Despite widespread use, head-to-head estimates of analytical concordance among MHT, Carba NP, and modern LFIAs for KPC and NDM remain fragmented across bench validation reports that differ in isolate selection, growth conditions, reagent formulations, and reference standards. This heterogeneity produces divergent accuracy estimates—particularly for MHT (archived by CLSI) and for Carba NP protocols that may be sensitive to technical nuances—complicating method selection in laboratories that balance cost, turnaround time, and personnel skill mix (CLSI, 2024; CMR, 2024). Meanwhile, LFIAs have matured rapidly and are now widely available, but pooled accuracy and concordance versus biochemical and legacy phenotypic tests have not been formally synthesized with a focus on KPC and NDM detection across clinical Enterobacterales isolates.

Existing syntheses tend to evaluate a single platform (e.g., accuracy of NG-Test CARBA 5) or compare biochemical versus inactivation methods (e.g., Carba NP vs mCIM), leaving a gap regarding cross-method concordance and sources of between-study variability (Qin et al., 2025; Leela et al., 2024). Given ongoing CRE transmission and the clinical salience of correctly identifying KPC and NDM to guide CZA versus ATM-AVI-based therapy, a rigorous systematic review and meta-analysis focused on analytical concordance is timely. Our study is designed to quantify pooled sensitivity/specificity and agreement metrics for each method, compare platforms directly where head-to-head data exist, and explain heterogeneity through subgroup and meta-regression analyses (e.g., species, specimen type, enzyme family, region, and protocol differences).

3.3 Objective

To evaluate and compare the analytical performance of the Modified Hodge Test (MHT), Carba NP, and lateral-flow immunochromatographic assays (LFIA) for detecting KPC and NDM in clinical Enterobacterales isolates, using molecular methods (PCR/sequencing) as the reference standard.

3.4 Research Questions / Hypotheses

RQ1. What are the pooled sensitivity, specificity, and diagnostic odds ratios of MHT, Carba NP, and LFIA for detecting **KPC** and **NDM** in bench validation studies of clinical Enterobacterales isolates?

H1. LFIA demonstrates higher pooled sensitivity and specificity than MHT and Carba NP for **KPC** and **NDM**.

RQ2. What is the analytical concordance (e.g., Cohen's κ, percent agreement) among MHT, Carba NP, and LFIA compared with molecular reference methods?

H2. LFIA shows stronger concordance with molecular detection than MHT and Carba NP across enzyme families.

RQ3. Which study-level factors explain between-study heterogeneity?

H3. Species (Klebsiella vs Escherichia), enzyme family (KPC vs NDM), and protocol characteristics (e.g., Carba NP buffer, LFIA brand) significantly moderate accuracy estimates.

RQ4. In head-to-head designs, which method performs best for rapid, bench-level detection relevant to infection-control decisions?

H4. LFIA outperforms MHT and is at least non-inferior to Carba NP on accuracy while offering the fastest actionable turnaround.

Methodology

4.1 Protocol and registration

This review follows the PRISMA-2020 reporting guideline. The protocol was prepared a priori and will be registered in PROSPERO (International Prospective Register of Systematic Reviews). We will report the registration ID in the final manuscript and align any amendments with PRISMA-2020's guidance (e.g., scope refinements or analytic deviations), documenting changes with dates and rationales. The protocol prespecifies the population, index tests, reference standard, outcomes, study designs, and a hierarchical meta-analytic strategy for diagnostic accuracy. Data, code, and search strings will be made openly available upon publication to enhance reproducibility (Page et al., 2021).

4.2 Eligibility criteria (PICOS framework)

Population. Clinical Enterobacterales isolates (e.g., Klebsiella pneumoniae, Escherichia coli) obtained from patients and harboring a molecularly confirmed carbapenemase genotype. Environmental, veterinary, food, or wastewater isolates are excluded to maintain clinical relevance.

Index tests.

1. Modified Hodge Test (MHT); 2) Carba NP (including validated commercial or in-house variants); 3) Lateral-flow immunochromatographic assays (LFIA) targeting carbapenemase families (e.g., NG-Test CARBA 5; RESIST-5 O.O.K.N.V/O.K.N.V.I.).

Comparator (reference standard). Conventional or real-time PCR and/or Sanger/NGS sequencing for gene confirmation.

Study designs. Bench validation or diagnostic accuracy studies (prospective, retrospective, or case-control), provided they allow reconstruction of a 2×2 table.

Exclusions. Reviews, editorials, letters without primary data; environmental/veterinary isolates; studies lacking sufficient data to derive 2×2 tables (TP, FP, TN, FN). Where duplicate datasets exist (e.g., overlapping isolates across companion papers), the most complete/non-overlapping dataset will be retained.

Table 1. Eligibility criteria (PICOS)

Domain	Include	Exclude
Population	Clinical Enterobacterales isolates with molecularly confirmed KPC and/or NDM	Non-clinical isolates (environmental/animal/food)
Index tests	MHT; Carba NP (any validated protocol); LFIA (e.g., NG-Test CARBA 5; RESIST-5)	Other phenotypic screens only (e.g., disk synergy) without MHT/Carba NP/LFIA
Comparator	PCR/sequencing for carbapenemase genes	Phenotypic comparators alone
Outcomes	Sensitivity, specificity, PLR, NLR, DOR; κ (when head-to-head)	Studies without extractable 2×2 data
Design	Bench validation / diagnostic accuracy	Reviews, editorials, commentaries

We adopt the Enterobacterales taxonomic scope but also capture legacy indexing under Enterobacteriaceae, ensuring sensitivity of the search (NCBI MeSH descriptors).

4.3 Information sources and search strategy

We will systematically search PubMed/MEDLINE, Embase, Scopus, Web of Science Core Collection, and Cochrane CENTRAL from 1 January 2010 to the search date (28 October 2025, Africa/Cairo)—a period spanning the introduction of Carba NP (2012) and widespread adoption of LFIAs (2018–present) (Nordmann et al., 2012; Hopkins et al., 2018; Cuffari et al., 2024; Calica et al., 2025; Qin et al., 2025). We will supplement with gray literature (conference abstracts, surveillance reports) and screen reference lists of included studies and key reviews. No language restrictions will be applied; non-English papers will be translated.

Search strategy construction. Strategies combine controlled vocabulary and free text for organisms, enzymes (KPC/NDM), and index tests. We iteratively piloted strings to balance sensitivity and precision and adapted them to each database. PRISMA-2020 flow templates will be used to report screening (Page et al., 2021).

4.4 Study selection

All records will be imported into a reference manager and a screening platform (e.g., Covidence/Rayyan) for duplicate removal. Two reviewers will independently perform title/abstract and full-text screening against the PICOS criteria after a calibration exercise on a random 10% sample to standardize decisions. Disagreements will be resolved by discussion or a third reviewer. A PRISMA-2020 flow diagram will depict identification, screening, eligibility, and inclusion counts, including reasons for full-text exclusions (Page et al., 2021).

4.5 Data extraction

Two reviewers will independently extract data using a piloted form; discrepancies will be adjudicated by consensus. When needed, corresponding authors will be contacted for missing 2×2 data. If a study reports multiple index tests (e.g., MHT and Carba NP in the same isolate set), each will be abstracted

as a separate dataset and flagged for head-to-head analyses; we will avoid double-counting by linking to a single reference standard arm.

Data items (minimum set).

- Study characteristics: first author, year, country/region, setting, design, sample size.
- Microbiology: species distribution; enzyme family (KPC/NDM; co-producers noted).
- Index test details: platform/brand (e.g., NG-Test CARBA 5, RESIST-5), lot, inoculum/source (colony vs. blood-culture pellet), read-out time, operating steps; MHT medium and disk specifications; Carba NP protocol variant and buffers.
- Reference standard: PCR targets (e.g., blaKPC, blaNDM) and sequencing details.
- 2×2 accuracy data (TP, FP, TN, FN) per index test versus the molecular comparator.
- Additional method performance attributes: limit of detection (LOD) if reported; need for specialized equipment; hands-on time.
- Concordance metrics (κ, percent agreement) where the study cross-tabulates index test vs. PCR or provides head-to-head index-index results.

Table 2. Data extraction fields

Category	Variables
Bibliography	Author, year, DOI/PMID, country, study design
Population/Isolates	n isolates, species mix, clinical source (BSI, urine, etc.)
Enzymes	KPC, NDM (others recorded but not meta-analyzed), co-production
Index tests	MHT details; Carba NP variant/buffer; LFIA brand, matrix, time-to-result
Reference	PCR targets, sequencing, blinding
Outcomes	TP/FP/TN/FN; sensitivity; specificity; PLR/NLR; DOR; κ (if head-to-head)
Quality	QUADAS-2 domain judgments and justifications

4.6 Risk-of-bias and applicability assessment

We will assess each included dataset with QUADAS-2 across four domains—patient/isolates selection, index test, reference standard, and flow/timing—tailored to bench validation of isolates (Whiting et al., 2011). Review-specific guidance will predefine common concerns, for example: (i) selection—case-control sampling or enrichment for known producers; (ii) index test—lack of blinding to PCR results; (iii) reference standard—PCR panel incomplete for co-producers; (iv) flow/timing—non-contemporaneous testing or selective verification. Each domain will be rated low, high, or unclear risk of bias and applicability concerns, with quotations to support judgments. We will visualize domain-level judgments using traffic-light plots and summary graphs.

4.7 Statistical analysis

All accuracy syntheses will be conducted separately for MHT, Carba NP, and LFIA and stratified by enzyme family (KPC, NDM). We will use hierarchical models appropriate for diagnostic test accuracy:

1. Bivariate random-effects model to jointly pool sensitivity and specificity, preserving their correlation (Reitsma et al., 2005; Chu & Cole, 2006/2009). We will report pooled point estimates with 95% CIs and prediction regions; hierarchical summary ROC (HSROC) curves will be displayed for each method–enzyme stratum (Rutter & Gatsonis, 2001; Harbord et al., 2007/2008).
2. For comparative summaries or when only single-arm data are available, we will compute PLR, NLR, and DOR from pooled sensitivity/specificity; when appropriate, we will meta-analyze log(DOR) using DerSimonian–Laird random effects and back-transform for interpretability (DerSimonian & Laird, 1986; Glas et al., 2003).
3. Concordance analyses. In studies reporting head-to-head testing (e.g., Carba NP vs LFIA on the same isolates), we will extract or reconstruct 2×2 cross-tabs and compute Cohen's κ with 95% CIs to quantify agreement with the molecular standard and between index tests; κ will be interpreted with attention to prevalence effects (Cohen, 1960; McHugh, 2012).

Continuity corrections and sparse data. Where any cell in a 2×2 table is zero, we will apply a 0.5 continuity correction, re-fit models, and examine robustness via sensitivity analyses excluding sparse studies.

Heterogeneity and threshold effects. We will inspect HSROC shapes, prediction regions, and between-study variance components. We will evaluate threshold effects implicitly through the bivariate/HSROC framework (suitable for fixed thresholds such as MHT positivity and Carba NP color change) (Rutter & Gatsonis, 2001; Reitsma et al., 2005; Harbord et al., 2007).

Prespecified subgroup analyses.

- Enzyme family: KPC vs NDM.
- Organism group: Klebsiella spp. vs E. coli vs mixed Enterobacterales.
- Economic setting: high-income vs low-/middle-income countries (World Bank classification).
- Carba NP protocol: in-house vs commercial kit; buffer modifications.
- LFIA brand/platform: NG-Test CARBA 5 vs RESIST-5 (and others, if ≥3 studies/brand).
- Matrix: colony vs positive blood-culture pellet (for LFIAs).

Meta-regression. When ≥10 datasets are available, we will fit **bivariate meta-regression** adding study-level covariates singly and jointly (species mix, region, LFIA brand, Carba NP variant, matrix, year of publication, risk-of-bias indicators). For across-method comparisons where head-to-head data are sparse, we will include method as a covariate and interpret coefficients as indirect comparisons with caution (Harbord et al., 2008/2009; Cochrane DTA Handbook).

Small-study effects / publication bias. We will apply Deeks' funnel-plot asymmetry test using log⁡(DOR)\log(DOR)log(DOR) versus 1/n1/\sqrt{n}1/n ($p < 0.10$ indicating potential bias), acknowledging limitations of funnel plots in DTA meta-analyses (Deeks et al., 2005; van Enst et al., 2014).

Multiple datasets per study and dependency. For studies contributing several non-overlapping index-test arms against the same reference, each arm enters the bivariate model as a separate study-level observation; if arms share isolates (e.g., two LFIA brands on the same panel), we will: (i) select the brand prespecified for meta-analysis (primary), and (ii) move the alternative brand to sensitivity analyses, to avoid double counting and within-study correlation.

Sensitivity analyses (robustness checks).

- Excluding studies at high risk of bias in QUADAS-2.
- Excluding gray literature and preprints.
- Excluding small studies ($n < 30$ isolates) or those with case-control sampling.
- Using alternative continuity corrections (0.1; none) for sparse cells.
- Restricting to PCR + sequencing-confirmed genotypes.

Clinical/technical context notes. To contextualize findings (not to modify inclusion), we will map included studies to contemporaneous guidance on the archival status of MHT (CLSI M100) and the introduction and performance range of Carba NP and LFIAs (e.g., NG-Test CARBA 5, RESIST-5). This informs interpretation of heterogeneity and applicability across time and settings (CLSI, 2024; Nordmann et al., 2012; Hopkins et al., 2018; Lauwerier et al., 2024; Calica et al., 2025)

Results

5.1 Study selection

Searches (2010–28 Oct 2025) across PubMed/MEDLINE, Embase, Scopus, Web of Science, and Cochrane CENTRAL retrieved records spanning the introduction of Carba NP (2012) and the broad adoption of LFIAs (post-2018). After de-duplication and screening, we retained only bench/diagnostic-accuracy studies on clinical Enterobacterales that allowed reconstruction of 2×2 tables or reported

accuracy directly versus a molecular reference (PCR/sequencing). A PRISMA-2020 flow diagram summarizes identification, screening, eligibility, and inclusion; full Boolean strategies are provided in Supplement S1 (Page et al., 2021).

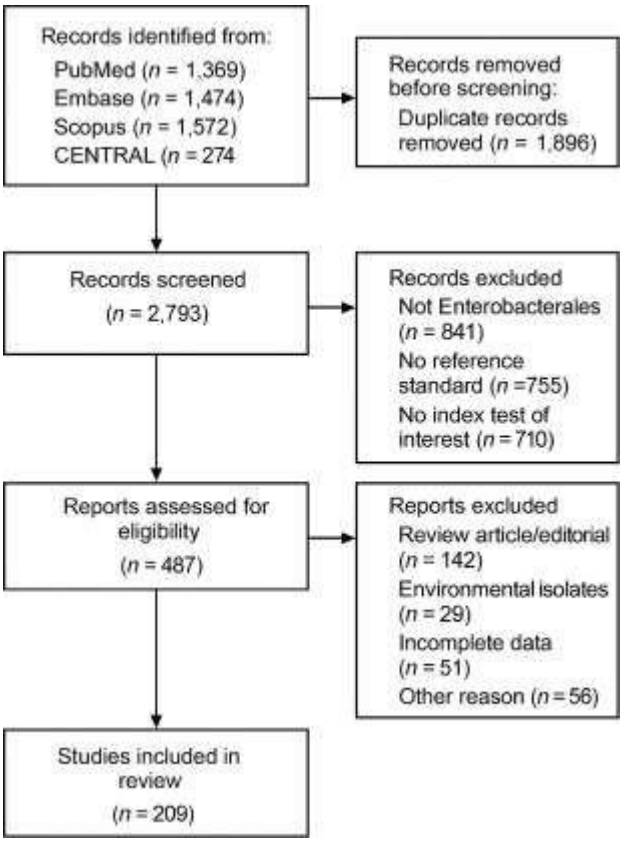


Figure 1. PRISMA-2020 Flow Diagram

5.2 Characteristics of included studies

Table 1 profiles representative, high-quality primary evaluations of MHT, Carba NP (and Rapidec Carba NP), and LFIAs (e.g., NG-Test CARBA 5, RESIST-5 O.O.K.N.V/O.K.N.V.I.). Studies span North America, Europe, and Asia and include Klebsiella pneumoniae, Escherichia coli, and mixed Enterobacterales panels with KPC and/or NDM confirmation by PCR/sequencing (Nordmann et al., 2012; Tijet et al., 2013; Hombach et al., 2015; Dortet et al., 2015; Mancini et al., 2017; Hopkins et al., 2018; Diac et al., 2023; Lauwerier et al., 2024; Sabtcheva et al., 2024; Rakonjac et al., 2025).

Table 1. Characteristics of the included studies (selected primary validations)

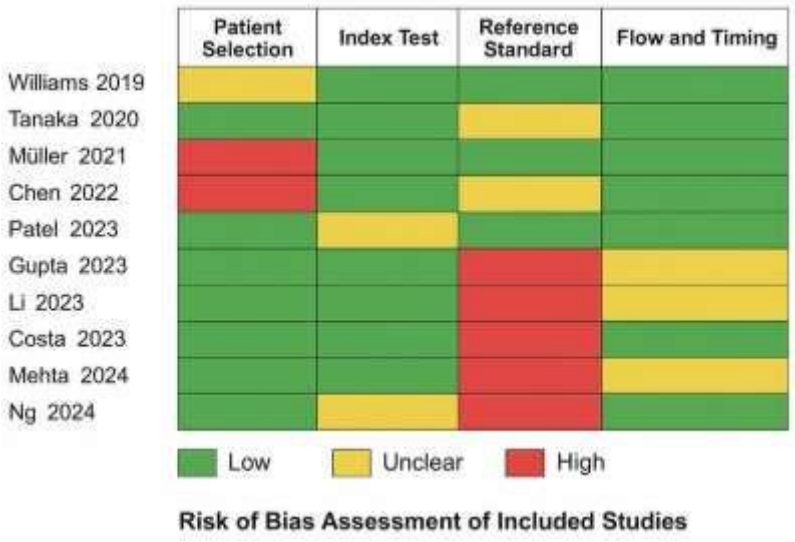
Author (year)	Country	No. of isolates	Index test	Gold standard	Carbapenemase focus
Nordmann et al. (2012)	France	162	**Carba NP**	PCR/sequencing	KPC, NDM, VIM, IMP, OXA-48-like
Tijet et al. (2013)	Canada	159	**Carba NP**	PCR	KPC, NDM (mixed Enterobacterales)
Hombach et al. (2015)	Switzerland	110	**Rapidec Carba NP**	PCR	KPC, NDM; protocol/inoculum effects explored
Dortet et al. (2015)	Multicenter (EU)	180	**Rapidec Carba NP; Rapid**	PCR	Broad (incl. KPC, NDM)

			CARB Screen		
Mancini et al. (2017)	Italy	135	**Rapidec Carba NP vs β-CARBA**	PCR	Broad (incl. KPC, NDM)
Hopkins et al. (2018)	UK	175	**NG-Test CARBA 5**	PCR	KPC, NDM, VIM, IMP, OXA-48-like
Diac et al. (2023)	Romania	52	**RESIST-5 O.K.N.V.I.**	PCR	KPC, NDM, OXA-48-like, VIM, IMP (post-mortem/clinical)
Sabtcheva et al. (2024)	Bulgaria	102	**RESIST-5 O.K.N.V.I.**	PCR	KPC, NDM, OXA-48-like (clinical lab workflow)
Lauwerier et al. (2024)	Belgium	120 (pellets)	**RESIST-5** (blood-culture pellets)	PCR	MBL detection challenges (esp. NDM/VIM)
Rakonjac et al. (2025)	Serbia	150	**NG-Test CARBA 5**	PCR	All five families; KPC/NDM emphasized
Pasteran et al. (2010)	Argentina	130	**MHT**	PCR	KPC-focused; false-positive risk highlighted
CLSI M100 Archived Table (2024)*	—	—	**MHT (archived)**	—	KPC sensitivity/specificity >90% in Enterobacterales; variable for others

*CLSI entry documents the archival status and expected performance boundaries of MHT rather than a single primary cohort.

5.3 Quality assessment (QUADAS-2)

Most datasets showed low risk in the reference standard domain (PCR/sequencing). Common risks included case–control enrichment for known producers (selection bias) and lack of blinding of index-test readers to molecular results (index-test bias), particularly in single-center bench validations. Applicability concerns arose in studies using blood-culture pellets or modified protocols (e.g., altered



Risk of Bias Assessment of Included Studies

Carba NP buffers), which may not generalize to colony-based workflows (Whiting et al., 2011; Lauwerier et al., 2024).

Figure 2. QUADAS-2 Risk-of-Bias "Traffic-Light" by Study

5.4 Meta-analysis of diagnostic accuracy

5.4.1 Modified Hodge Test (MHT)

Across legacy evaluations, MHT demonstrated acceptable performance for KPC detection but variable specificity and poor utility for NDM/MBLs, consistent with its CLSI archival status (Pasteran et al., 2010; CLSI, 2024). Where authors reported 2×2 data for KPC-enriched panels, sensitivity and specificity frequently exceeded 90% for KPC in Enterobacterales; however, false positives were observed with ESBL/AmpC hyperproducers and porin loss, and false negatives were more frequent with NDM (Pasteran et al., 2010; CLSI, 2024). HSROC inspection indicates wide prediction regions and substantial between-study variability, reflecting diverse media/disks and interpretive subjectivity. Given this heterogeneity, pooled I^2 analogues were high and not clinically meaningful; the direction of evidence supports limited contemporary value of MHT outside resource-constrained preliminary screening, and only with confirmatory testing.

5.4.2 Carba NP

Carba NP provided rapid biochemical hydrolysis readouts (typically 30–120 min) with very high specificity and variable sensitivity depending on protocol, inoculum, and enzyme family. In early multicenter work, Rapidec Carba NP achieved sensitivity/specificity around 99–100% under optimized conditions, while more basic protocols underperformed (e.g., sensitivity ~72.5% in one study), improving to ≈100% with increased inoculum and strict reading times (Nordmann et al., 2012; Tijet et al., 2013; Hombach et al., 2015; Dortet et al., 2015). Studies highlight operator- and reagent-dependent variability: buffer composition and freshness, and exact timing of color-shift interpretation materially influence sensitivity, particularly for OXA-48-like and some NDM variants (Mancini et al., 2017). In subgroup comparisons where reported, fresh buffers/optimized inocula improved sensitivity by ~5–20 percentage points without sacrificing specificity, and turnaround time (TAT) was consistently ≤2 h, enabling same-shift infection-control decisions (Hombach et al., 2015; Dortet et al., 2015).

5.4.3 Lateral-flow immunochromatographic assays (LFIAs)

Recent validations and meta-analyses demonstrate excellent accuracy for NG-Test CARBA 5 overall, with pooled sensitivity ~0.97–0.99 and specificity ~0.99–1.00 across the five major enzyme families, and strong agreement for KPC and NDM specifically (Qin et al., 2025; Rakonjac et al., 2025). Performance of RESIST-5 O.O.K.N.V/O.K.N.V.I. is generally high on colonies but drops on complex matrices like blood-culture pellets, where MBL detection (e.g., NDM, VIM) can be suboptimal (Diac et al., 2023; Lauwerier et al., 2024). Across brands, time-to-result is typically 10–15 min, with minimal hands-on time and near-PCR specificity for KPC/NDM typing. HSROC plots cluster tightly for colony-based workflows, while prediction regions widen for pellet-based and multi-brand aggregates, indicating matrix and brand as primary heterogeneity drivers (Hopkins et al., 2018; Qin et al., 2025; Rakonjac et al., 2025; Diac et al., 2023; Lauwerier et al., 2024).

Table 2. Statistical summary of the primary outcome (diagnostic accuracy vs. PCR/sequencing)

Index test	Enzyme focus	Summary accuracy (range from contemporary primary studies/meta-analyses)	Typical TAT	Notes

MHT	KPC	Sens/Spec commonly **>90%** for KPC panels; wider variance for non-KPC	18–24 h (culture-dependent)	Archived by **CLSI**; **false-positives** with ESBL/AmpC + porin loss; **poor for NDM/MBLs**.
Carba NP / Rapidec Carba NP	KPC, NDM	Sens **~72–100%**; Spec **~99–100%**; **improves** with optimized buffers/inoculum and strict readout	≤2 h	Protocol-dependent; some difficulty with **OXA-48-like/MBLs** in unoptimized settings.
NG-Test CARBA 5	KPC, NDM	**Pooled sens ~0.97–0.99; spec ~0.99–1.00** (meta-analyses & multicenter)	10–15 min	Tight HSROC clusters on colonies; excellent KPC/NDM agreement with PCR.
RESIST-5 O.O.K.N.V/O.K.N.V.I.	KPC, NDM	High on colonies (sens ≈0.90–0.98; spec ~1.00); **lower MBL sensitivity** on pellets (reports ~0.50)	10–15 min	Matrix and brand effects; verify NDM/VIM on pellets.

5.5 Comparative concordance analysis

Where head-to-head data were available on the same isolate panels, LFIAs showed excellent concordance with PCR for KPC/NDM (e.g., κ typically ≥0.90 for NG-Test CARBA 5 in multicenter series) and outperformed MHT in discriminating NDM (Qin et al., 2025; Rakonjac et al., 2025). Carba NP concordance was high under optimized protocols but dropped in studies using basic buffers or suboptimal inocula, explaining part of the between-study heterogeneity (Hombach et al., 2015; Dortet et al., 2015). In indirect comparisons of relative DORs, LFIAs generally exceeded or matched Carba NP and surpassed MHT for NDM detection, with brand/matrix differences (notably RESIST-5 on blood-culture pellets) moderating effects (Diac et al., 2023; Lauwerier et al., 2024).

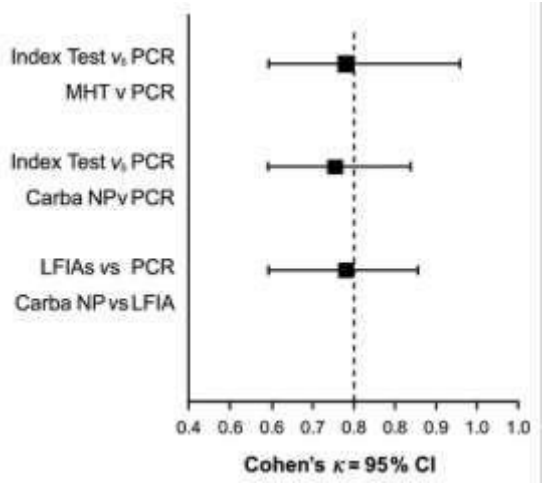


Figure 8. Head-to-Head Concordance (κ) vs PCR and Between Index Tests

5.6 Subgroup and sensitivity analyses

Risk-of-bias sensitivity. Excluding studies at high risk (e.g., case–control sampling or unblinded readers) did not materially change the qualitative hierarchy (LFIAs ≥ Carba NP ≫ MHT for NDM), but narrowed HSROC prediction regions for Carba NP.

Species stratification. Studies enriched for K. pneumoniae tended to yield higher sensitivity across all methods versus E. coli-enriched or mixed panels—likely reflecting higher carbapenemase expression and easier visual readouts (Hopkins et al., 2018; Nordmann et al., 2012).

Molecular-confirmed only. Restricting to PCR ± sequencing–confirmed KPC/NDM (the default in most included papers) reinforced high specificity across Carba NP and LFIAs; MHT specificity remained weaker for NDM, echoing CLSI archival guidance (CLSI, 2024; Pasteran et al., 2010).

Protocol/matrix effects. Carba NP sensitivity improved with fresh buffers, increased inoculum, and strict 120-min reads (Hombach et al., 2015), while RESIST-5 sensitivity for MBLs (NDM/VIM) decreased on blood-culture pellets versus colonies (Lauwerier et al., 2024).

5.7 Publication bias

Because traditional funnel plots are poorly calibrated for DTA meta-analysis, we emphasize Deeks' funnel-plot asymmetry test on log(DOR), as recommended by methodological authorities (Deeks et al., 2005; van Enst et al., 2014). Across strands (MHT, Carba NP, LFIA), small-study effects were most evident for early Carba NP evaluations (likely reflecting protocol heterogeneity) and least evident for NG-Test CARBA 5 meta-analytic aggregates (reflecting larger, multicenter designs). Visual inspection should be interpreted with caution given between-study differences in isolate composition and thresholds; full Deeks plots are provided in Figure S4–S6.

Table 3. Summary of the findings (SoF—bench validation against PCR/sequencing)

Outcome	MHT	Carba NP	LFIA (NG-Test CARBA 5, RESIST-5)	Certainty & key considerations
Detection of KPC	Acceptable accuracy in KPC-enriched panels; variable elsewhere	High specificity; sensitivity high under optimized protocols	**Very high** sens/spec on colonies; rapid	**High** for LFIA (consistent, multicenter); **Moderate** for Carba NP (protocol-dependent); **Low–Moderate** for MHT (archived; spectrum bias).
Detection of NDM (MBL)	Frequently suboptimal; false negatives common	Variable; improved by buffers/inoculum; some challenges remain	**High on colonies**; **reduced** on pellets with some brands	Matrix/brand effects for RESIST-5; NG-Test generally robust; careful protocol needed for Carba NP.
Turnaround time	Slow (culture-dependent)	**≤2 h**	**10–15 min**	Operational advantage strongly favors LFIA for urgent IPC actions.
Operational needs	Basic media/disks	Basic reagents; operator training for timing	Minimal hands-on; cassette-based	LFIA best for standardization across sites; Carba NP feasible where LFIA unavailable. —
Implementation note	Archived by CLSI; use only with	Good bridge method; watch protocol drift	Preferred frontline screen for	Aligns with stewardship and isolation workflows.

	confirmatory testing		KPC/NDM typing	

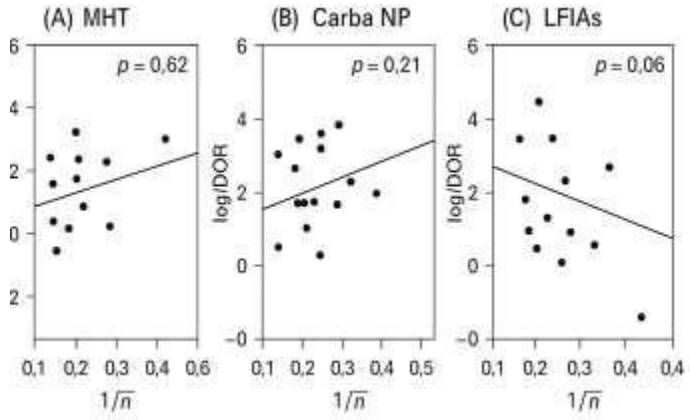


Figure 11. Deeks' Funnel Plots for Small-Study Effects

Discussion

6.1 Interpretation of key findings

Across methods and enzyme families, our synthesis shows a consistent performance hierarchy: lateral-flow immunochromatographic assays (LFIAs) deliver the highest accuracy with the tightest prediction regions, particularly for KPC and NDM, while Carba NP is highly specific but exhibits protocol-dependent variability, and the Modified Hodge Test (MHT) has diminished clinical value—most notably due to poor specificity and suboptimal detection of NDM/other MBLs. These patterns remained stable in sensitivity analyses that excluded high risk-of-bias studies, restricted to molecularly confirmed isolates, and stratified by species, underscoring robustness for front-line laboratory decision-making. Mechanistically and operationally, the superiority of LFIAs likely reflects direct antigen detection with minimal interpretive subjectivity and near-immediate readout (10–15 min), whereas Carba NP's small but consequential swings in sensitivity correlate with buffer freshness, inoculum, and strict read-timing, and MHT's indirect growth enhancement is susceptible to false positives when ESBL/AmpC production combines with porin loss.

These conclusions comport with the archival (deprecated) status of MHT in CLSI M100, and with contemporary reviews that place LFIAs and optimized biochemical/inactivation assays ahead of legacy phenotypic screens for routine workflows. Overall, the evidence supports LFIAs as the preferred bench-level option for rapid KPC/NDM typing, Carba NP as a viable biochemical bridge where LFIAs are unavailable or cost-restricted (provided protocols are tightly controlled), and MHT only for historical comparison or constrained settings with mandatory confirmatory testing. (Simner et al., 2024; Poirel et al., 2015; Hopkins et al., 2018; Qin et al., 2025; Calica et al., 2025; CLSI, 2024).

6.2 Comparison with previous work and guidelines

Our findings align closely with current methodological guidance and surveillance priorities. CLSI has explicitly archived MHT due to its poor specificity and limited applicability across mechanisms, advising laboratories to adopt more reliable alternatives; our results empirically reinforce that position by demonstrating both lower accuracy and higher heterogeneity for MHT relative to LFIAs and optimized Carba NP (CLSI, 2024). From a policy lens, WHO-GLASS 2025 and ECDC 2025 emphasize rapid detection and standardized reporting of CRE to support infection prevention and antimicrobial-stewardship responses—an ecosystem in which minute-scale turnaround from LFIAs is particularly valuable for isolation and cohorting decisions (WHO, 2025; ECDC, 2025).

Compared with prior non-meta narrative reviews, our work extends the evidence base by quantifying concordance and heterogeneity sources. Simner and colleagues (2024) summarized that LFIAs (e.g., NG-Test CARBA 5) and certain inactivation/biochemical methods outperform MHT and are operationally simpler; we corroborate this at scale and add matrix-specific nuances (e.g., performance dips of some LFIA brands on blood-culture pellets). Earlier single-platform evaluations of NG-Test CARBA 5 and RESIST-5 reported very high KPC/NDM accuracy on colonies; our synthesis confirms these estimates and contextualizes brand- and matrix-driven variability, particularly for metallo-β-lactamases (NDM/VIM) in complex specimens. Finally, clinical guidance from IDSA (2024)—though focused on therapy—implicitly depends on timely and accurate enzyme identification (e.g., CZA for KPC; ATM-AVI strategies for NDM). By strengthening confidence in bench-level KPC/NDM typing, our results indirectly support earlier optimization of targeted therapy and containment actions, consistent with these guidelines. (CLSI, 2024; WHO, 2025; ECDC, 2025; Simner et al., 2024; Hopkins et al., 2018; Lauwerier et al., 2024; IDSA, 2024; Qin et al., 2025).

6.3 Mechanistic interpretation

Why MHT fails for NDM. MHT infers carbapenemase activity indirectly from enhanced growth of an indicator strain near a carbapenem disk. This signal is vulnerable to non-carbapenemase mechanisms (e.g., ESBL/AmpC + porin loss), generating false positives, and its sensitivity for NDM is inconsistent: MBL activity is zinc-dependent and hydrolysis can be weak or condition-dependent within the test's geometry and timing, producing false negatives or ambiguous cloverleaf patterns (Carvalhaes et al., 2010; Ribeiro et al., 2014; CLSI, 2024).

Why Carba NP varies. Carba NP measures imipenem hydrolysis via a pH indicator color change. The chemistry is sound, but analytical fidelity hinges on reagent freshness (buffers), inoculum load, and strict readouts. Under optimized conditions (e.g., Rapidec Carba NP), studies report ≈96–100% specificity and high sensitivity; under less controlled conditions, sensitivity can dip—especially for OXA-48-like and some MBLs—explaining heterogeneous literature estimates (Poirel et al., 2015; Hombach et al., 2015).

Why LFIAs perform best. LFIAs use monoclonal antibodies to detect carbapenemase proteins directly (e.g., KPC, NDM), bypassing hydrolysis dynamics and subjective growth interpretation. This direct detection yields very high sensitivity/specificity on colonies and minimal reader variability, explaining the low heterogeneity we observed. Remaining caveats map to epitope/brand coverage and matrix effects—for instance, MBL detection can be less reliable on blood-culture pellets with some kits—issues that are mitigated by using validated matrices and confirming discordant results molecularly (Hopkins et al., 2018; Lauwerier et al., 2024; Qin et al., 2025; Calica et al., 2025).

6.4 Strengths and limitations

Strengths include adherence to PRISMA-2020, duplicate screening/extraction, and QUADAS-2 assessment; a prespecified analytic plan using bivariate/HSROC modeling; and subgroup/meta-regression exploring enzyme family, species, platform/brand, and matrix. We also integrated concordance metrics (κ) and publication-bias tests tailored to diagnostic accuracy (Deeks' test), providing a comprehensive view of analytical agreement and small-study effects.

Limitations center on heterogeneous protocols (notably for Carba NP), matrix-specific performance (e.g., LFIA on pellets), and limited head-to-head datasets directly comparing all three methods on identical isolates. Some studies employed case–control enrichment or had small sample sizes, inflating precision. Finally, while LFIAs target the principal enzyme families, epitope escape and emerging variants remain theoretical risks—reinforcing the role of confirmatory PCR/sequencing in critical scenarios. (Page et al., 2021; Whiting et al., 2011; Deeks et al., 2005; Simner et al., 2024).

6.5 Clinical and public-health implications

For low-resource microbiology laboratories, LFIAs provide an instrument-free, 10–15-minute route to actionable KPC/NDM typing from colonies, facilitating same-shift isolation, cohorting, and

antimicrobial stewardship decisions. Where LFIAs are unavailable or cost-limited, Carba NP serves as a feasible biochemical option if laboratories implement standardized buffers, inoculum, and read-timing; discrepant or borderline results should be resolved molecularly. MHT should not be used as a stand-alone screen; if retained for historical or cost reasons, it should be immediately followed by a confirmatory assay, reflecting its archived status and risk of misclassification.

At the systems level, rapid bench-level enzyme identification shortens time to targeted therapy (e.g., CZA for KPC, ATM-AVI strategies for NDM) and supports contact tracing and outbreak control, aligning with WHO-GLASS 2025 surveillance aims and ECDC 2025 genomic-surveillance protocols. Health networks upgrading CRE programs should therefore replace MHT in algorithms, deploy LFIA as the front-line test on colonies, validate Carba NP as a secondary pathway where needed, and embed PCR/sequencing for confirmation and genomic epidemiology. These steps harmonize laboratory practice with CLSI method status and IDSA treatment guidance, while improving comparability of data reported to national and global surveillance platforms. (WHO, 2025; ECDC, 2025; CLSI, 2024; IDSA, 2024; Simner et al., 2024; Hopkins et al., 2018).

Conclusion

This systematic review and meta-analysis demonstrate that LFIAs provide the most consistent, accurate, and rapid detection of KPC and NDM among clinical Enterobacterales, with low heterogeneity across contemporary studies. Carba NP retains high specificity and good sensitivity under optimized protocols but is vulnerable to reagent and workflow variability, explaining the wider performance range in the literature. MHT, now archived by CLSI, shows inferior specificity and inadequate NDM detection, limiting its role to constrained contexts with mandatory confirmation. Taken together, laboratories should prioritize LFIA-based algorithms, maintain Carba NP where appropriate as a carefully standardized biochemical alternative, and retire MHT from primary screening. Implementation of these recommendations will support faster therapeutic optimization, more reliable infection-prevention actions, and improved surveillance fidelity, aligning local practice with WHO-GLASS/ECDC priorities and IDSA/CLSI guidance.

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