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Brief Report

Analytical Inclusivity of Three Commercial Real-Time PCR Platforms Against Carbapenemase Allelic Variants in Peruvian Clinical Isolates

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Abstract

Objectives: Rapid molecular detection of carbapenemase-producing Gram-negative bacilli is crucial for targeted antimicrobial therapy, but regional gene sequence diversity can impair commercial PCR assays. This study evaluated the analytical inclusivity of three commercial real-time PCR platforms (Xpert Carba-R on the GeneXpert system, Galaxy Lite, and Molecular Mouse) against carbapenemase alleles circulating in Peru, including the regionally relevant $bla_{\text{IMP-74}}$ and $bla_{\text{IMP-93}}$ alleles. **Methods**: We analyzed a positive-enriched challenge panel of 23 non-duplicate clinical isolates previously characterized by whole-genome sequencing (WGS): 10 *Enterobacterales* and 13 *Pseudomonas aeruginosa* carrying 26 carbapenemase genes across 12 variants ($bla_{\text{KPC-2/35}}$, $bla_{\text{NDM-1/5}}$, $bla_{\text{OXA-48/181/232}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{IMP-16/18/74/93}}$); inclusivity was calculated per WGS-confirmed target gene, and Molecular Mouse was evaluated on a 16-target subset including at least one representative of each allele. **Results**: Galaxy Lite detected all carbapenemase alleles evaluated (12/12 alleles; gene-level analytical inclusivity 100%, 26/26 target genes, 95% CI: 86.8–100%). Xpert Carba-R and Molecular Mouse each detected 8 of the 12 alleles (100% of KPC, NDM, OXA-48-like, and VIM alleles), failing to detect any of the four IMP alleles evaluated. Overall gene-level inclusivity was 53.8% (14/26 target genes, 95% CI: 33.4–73.4%) for Xpert Carba-R and 62.5% (10/16 target genes, 95% CI: 35.4–84.8%) for Molecular Mouse. **Conclusions**: In this small, positive-enriched panel, two of the three commercial PCR panels did not detect the IMP alleles tested, a finding that may be related to primer/probe–target sequence divergence, although this mechanism was not investigated. These results support the local verification of commercial carbapenemase PCR assays against circulating alleles before routine implementation.

Keywords: carbapenemases; real-time polymerase chain reaction; *Pseudomonas aeruginosa*; target drop-out; analytical inclusivity; IMP variants

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1. Introduction

The rapid global dissemination of carbapenem-resistant Gram-negative bacilli represents a critical threat to public health. In its 2024 Bacterial Priority Pathogens List, the World Health Organization (WHO) classified carbapenem-resistant *Enterobacterales* (CRE) as critical-priority pathogens and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) as a high-priority pathogen, the latter having been moved from the critical category of the previous list [1]. Both are associated with severe clinical impact, increased mortality, and

limited therapeutic options. Notably, carbapenem resistance is a phenotype that may result from several mechanisms, whereas carbapenemase production is one specific, frequently transferable mechanism of carbapenem resistance and is the focus of the present study. In clinical settings, rapid molecular detection of resistance mechanisms is essential for guiding targeted antimicrobial therapy and implementing infection control interventions to prevent nosocomial transmission [2].

In *Enterobacterales*, particularly *Klebsiella pneumoniae* and *Escherichia coli*, carbapenem resistance is frequently mediated by acquired carbapenemases of Ambler class A (e.g., KPC), class B (metallo-β-lactamases [MBLs], e.g., NDM, VIM, and IMP), and class D (OXA-48-like); however, it may also result from extended-spectrum β-lactamase (ESBL) or AmpC production combined with porin loss or decreased outer-membrane permeability [3]. In *P. aeruginosa*, carbapenem resistance frequently results from chromosomal mechanisms, such as OprD porin loss and overexpression of efflux systems (e.g., MexAB-OprM), rather than from carbapenemase production; in a multinational collection of 807 carbapenem-resistant *P. aeruginosa* isolates, only 33% were detected as carbapenemase-positive by the modified carbapenem inactivation method, with VIM being the most common enzyme, followed by GES, and the distribution of carbapenemases in this species varies widely by region [4]. In Peru, a WGS-based study of 141 carbapenemase-producing *P. aeruginosa* isolates from Lima exclusively identified IMP- and VIM-type enzymes [5].

Automated real-time PCR platforms allow rapid identification of major carbapenemase genes, reducing diagnostic turnaround times from days to hours. Phenotypic methods, such as the RAPIDEC CARBA NP test and the BD Phoenix CPO Detect test, can confirm carbapenemase activity but do not identify the specific carbapenemase gene or allele [6]. However, the analytical inclusivity of PCR assays depends on the conservation of the primer and probe binding sites within each target gene. Single primer–template mismatches within the 3′-end region of a primer can delay amplification by less than 1.5 to more than 7 quantification cycles, depending on the mismatch type and position [7]; multiple or strategically located mismatches may therefore reduce the fluorescence signal below the detection threshold and cause false-negative results. In this context, it is important to distinguish analytical inclusivity (the ability of an assay to detect the genetic diversity of its intended targets), analytical sensitivity (the lowest amount of target that can be reliably detected), and clinical sensitivity (the proportion of truly positive patients or specimens correctly identified). The IMP family is particularly challenging in this respect because it comprises a large and growing number of alleles distributed across divergent phylogenetic subgroups [8]. Accordingly, IMP target drop-out and false-negative results have been reported for commercial automated PCR assays, particularly where endemic alleles diverge from the sequences used in assay design [9,10].

In Peru, molecular surveillance has documented a dynamic landscape of carbapenemase variants (Table 1). In *Enterobacterales*, $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-1}}$ remain predominant. However, there are increasing reports of emerging variants, including $bla_{\text{NDM-5}}$, which may exhibit reduced susceptibility to cefiderocol [11]; plasmid-mediated $bla_{\text{OXA-48}}$-like genes; and the ceftazidime–avibactam-resistant $bla_{\text{KPC-35}}$ variant harboring an L169P omega-loop substitution [12]. Among carbapenemase-producing *P. aeruginosa*, the MBL gene $bla_{\text{VIM-2}}$ [13] and several bla_{IMP} alleles have been reported. In the WGS-based study from Lima mentioned above, ST111 (44.0%) and ST357 (38.3%) were the predominant lineages; 96.3% of ST357 isolates carried the then-novel $bla_{\text{IMP-93}}$ allele and the remainder carried $bla_{\text{IMP-74}}$; 74.2% of ST111 isolates co-harbored $bla_{\text{IMP-18}}$ and $bla_{\text{VIM-2}}$; and the remaining lineages carried $bla_{\text{IMP-16}}$, $bla_{\text{IMP-74}}$, or $bla_{\text{VIM-2}}$ [5]. IMP target drop-out in commercial assays has been described previously in Latin America and in international challenge collections [9,10,14]; however, to our knowledge, the performance of currently available platforms against the locally pre-

dominant $bla_{\text{IMP-93}}$ and $bla_{\text{IMP-74}}$ alleles has not been compared head-to-head, and data for the Galaxy Lite and Molecular Mouse platforms against these alleles are lacking. Therefore, we compared the analytical inclusivity of three commercial real-time PCR platforms using a challenge panel of Peruvian clinical isolates carrying 12 distinct carbapenemase alleles confirmed by whole-genome sequencing (WGS) [5,11,12,15].

Table 1. Alleles previously reported in Peru in published studies.

Variant	Bacterial Species	Healthcare Facility	Reference
KPC-2	*K. pneumoniae*	Cayetano Heredia Hospital	Krapp et al. (2025) [16]
	K. pneumoniae, *E. coli*	Arzobispo Loayza National Hospital	Cuicapuza et al. (2024) [17]
	K. pneumoniae, *E. coli*	Multicenter	Escalante et al. (2026) [11]
KPC-35	*K. pneumoniae*	Roe Laboratory	Gonzales-Rodriguez et al. (2025) [12]
NDM-1	*K. pneumoniae*, *E. coli*; *K. aerogenes*, *E. cloacae*	Arzobispo Loayza National Hospital	Cuicapuza et al. (2024) [17]
	K. pneumoniae	Cayetano Heredia Hospital	Krapp et al. (2025) [16]
	K. pneumoniae	Guillermo Almenara Irigoyen Hospital	Pons et al. (2020) [18]
	P. stuartii	Centenario Clinic	Lezameta et al. (2020) [19]
	K. pneumoniae, *E. coli*	Multicenter	Escalante et al. (2026) [11]
NDM-5	*E. coli*	Multicenter	Escalante et al. (2026) [11]
OXA-181	*K. pneumoniae*, *E. coli*, *Citrobacter portucalensis*	Multicenter	Cuicapuza et al. (2023) [15]
	K. pneumoniae	Multicenter	Escalante et al. (2026) [11]
OXA-48	*E. coli*	Multicenter	Escalante et al. (2026) [11]
OXA-232	*E. coli*	Multicenter	Tamariz et al. (in press)
VIM-2	*P. aeruginosa*	Naval Medical Center	Rios et al. (2018) [13]
	P. aeruginosa	Roe Laboratory	Tickler et al. (2022) [5]
IMP-16/18/74/93	*P. aeruginosa*	Roe Laboratory	Tickler et al. (2022) [5]

2. Materials and Methods

This retrospective analytical inclusivity study was conducted at Roe Laboratory in Lima, Peru, between May and December 2025. A challenge panel was purposively selected from the laboratory's archive of clinical isolates collected between 2018 and 2023 and previously characterized by whole-genome sequencing (WGS). The isolates were recovered from diverse clinical specimens, including urine, blood, respiratory tract secretions, and wound exudates. They were originally collected at Roe Laboratory as part of its participation in previously published multicenter genomic surveillance studies. The panel was specifically structured to include at least one representative of each carbapenemase allele documented in Peru and available within the repository. Isolates were considered non-duplicate when they originated from different patients, and only one isolate per patient was included. In total, 23 isolates were evaluated: *K. pneumoniae* ($n = 5$), *E. coli* ($n = 5$), and *P. aeruginosa* ($n = 13$). These isolates harbored 26 carbapenemase genes: 20 isolates carried a single gene and three carried two genes (1 *K. pneunoniae* $bla_{\text{NDM-1}}/bla_{\text{OXA-181}}$, 2 *P. aeruginosa* $bla_{\text{IMP-18/blaVIM-2}}$). The 26 genes corresponded to 12 distinct alleles of Ambler class A ($bla_{\text{KPC-2}}$ and $bla_{\text{KPC-35}}$), class B ($bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$, $bla_{\text{VIM-2}}$, $bla_{\text{IMP-16}}$, $bla_{\text{IMP-18}}$, $bla_{\text{IMP-74}}$, and $bla_{\text{IMP-93}}$), and class D (OXA-48-like: $bla_{\text{OXA-48}}$, $bla_{\text{OXA-181}}$, and $bla_{\text{OXA-232}}$). The number of targets per allele is shown in Table 2, and isolate-level data (species, sequence type, gene/allele content, and the result on each platform) are provided in Table S1. All isolates were previously characterized by WGS, which served as the reference standard [5,11,12,15].

Table 2. Analytical inclusivity of three commercial qPCR platforms against WGS-confirmed carbapenemase targets (unit of analysis: target gene).

Carbapenemase	Species	Allele	GeneXpert Xpert Carba-R	Galaxy Lite Carbapenem Resistance Kit	Molecular Mouse MM Gram Negative Resistance
KPC	Kp	KPC-2 KPC-35	100% (1/1) 100% (1/1)	100% (1/1) 100% (1/1)	100% (1/1) 100% (1/1)
NDM	Kp, Ec	NDM-1 NDM-5	100% (1/1) 100% (2/2)	100% (1/1) 100% (2/2)	100% (1/1) 100% (1/1)
OXA-48-like	Kp, Ec	OXA-48 OXA-181 OXA-232	100% (2/2) 100% (3/3) 100% (1/1)	100% (2/2) 100% (3/3) 100% (1/1)	100% (1/1) 100% (2/2) 100% (1/1)
VIM	Pa	VIM-2	100% (3/3)	100% (3/3)	100% (2/2)
IMP	Pa	IMP-16 IMP-18 IMP-74 IMP-93	0% (0/2) 0% (0/3) 0% (0/2) 0% (0/5)	100% (2/2) 100% (3/3) 100% (2/2) 100% (5/5)	0% (0/1) 0% (0/2) 0% (0/1) 0% (0/2)
Overall analytical inclusivity			53.8% (14/26) $p < 0.001$	100% (26/26)	62.5% (10/16) $p = 0.031$

Kp: *Klebsiella pneumoniae*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*. *p*-value: McNemar's test for paired data using the exact binomial distribution; pairwise comparisons were conducted against the Galaxy Lite platform. Molecular Mouse was tested on a predefined 16-target subset that included at least one representative of each allele; its overall estimate is therefore not directly comparable with those of the other two platforms. *p*-values are reported at the gene level; isolate-level sensitivity analyses are reported in the text. No carbapenemase-negative isolates were tested; specificity was not assessed.

Genomic DNA was sequenced on Illumina platforms (MiSeq/NextSeq; 2 × 150 bp or 2 × 250 bp paired-end reads) using Nextera XT DNA Library Prep kits. Raw reads were quality-trimmed using Trimmomatic v0.39 and assembled de novo with SPAdes v3.15.4 (mean depth of coverage ≥ 30x). Acquired carbapenemase genes were identified using ResFinder 4.1 (Center for Genomic Epidemiology), NCBI AMRFinderPlus (v3.11), and CARD with strict thresholds of ≥99% nucleotide identity and 100% gene coverage; allele assignment required an exact match over the full-length gene sequence. Sequence types (ST) were assigned using the MLST 2.0 tool according to PubMLST schemes. Genome accession numbers are listed in Table S1. Assays were performed using pure bacterial colonies grown for 18–24 h on 5% sheep blood agar. Isolates were recovered from frozen stocks (tryptic soy broth with 20% glycerol at −70 °C and subcultured twice on blood agar before testing to ensure purity and metabolic reactivation.

Three commercial multiplex real-time PCR platforms were evaluated according to the manufacturers' instructions: the Xpert Carba-R assay performed on the GeneXpert system (Cepheid, Sunnyvale, CA, USA), Carbapenem Resistance Diagnostic Kit executed on the Galaxy Lite system (Igenesis, Shanghai, China), and the MM Gram Negative Resistance assay on the Molecular Mouse platform (Alifax S.r.l., Polverara, Italy) (Table 3).

Each run included the internal controls specified by the manufacturer, and results were accepted only when these controls were valid. Operators performing the commercial assays were blinded to the WGS genotypes and alleles, and results were recorded as automatically reported by each platform's software, without manual interpretation of amplification curves. Assays were conducted in single testing without retesting negative results, provided that all internal amplification controls passed quality validation. Owing to the limited availability of commercial reagents during the study period, Molecular Mouse testing was restricted to a subset of 16 targets across 13 isolates, selected prior to testing to ensure representation of at least one isolate for each of the 12 identified alleles (Table 2 and Table S1).

Table 3. Specifications of the evaluated real-time PCR commercial platforms.

	GeneXpert	Galaxy Lite	Molecular Mouse
Manufacturer	Cepheid (USA)	Igenesis (China)	Alifax (Italy)
Assay	Xpert Carba-R	Carbapenem Resistance Kit	MM Gram Negative Resistance
Catalogue reference	GXCARBARP-CE-10	106-0102-01	SI 1701.0101
IFU	301.9242 Rev C Jun 2020.	Version A/2 October 2024	Version 1.6 2024-05-23
PCR Technology	Real-time PCR (Cartridge-based)	PCR-fluorescence (iCassette)	Real-time PCR (Lab-on-a-chip)
Carbapenemase targets	KPC, NDM, OXA-48-like, VIM, IMP	KPC, NDM, OXA-48-like, VIM, IMP	KPC, NDM, OXA-48-like, VIM, IMP, OXA-23

Target lists and assay information as stated in each manufacturer's instructions for use.

Analytical inclusivity was defined as the proportion of WGS-confirmed carbapenemase target genes detected by each platform. The primary unit of analysis was the target gene (26 gene detections in 23 isolates). Results were also summarized at the allele level (12 alleles) and at the isolate level, an isolate being considered correctly genotyped when all of its WGS-confirmed carbapenemase genes were detected. Because no carbapenemase-negative isolates were included, analytical specificity and overall diagnostic accuracy were not assessed. Corresponding 95% confidence intervals (95% CIs) were determined using the Clopper–Pearson exact method. Pairwise comparisons between platforms were performed with McNemar's exact test, restricted to targets tested on both platforms, with statistical significance set at $p < 0.05$. Because three isolates carried two carbapenemase genes, gene-level observations are not fully independent; therefore, the comparisons were repeated at the isolate level as a sensitivity analysis. Given the small and uneven number of targets per allele, *p*-values are interpreted descriptively. Analyses were performed with R software (version 4.5.1, R Foundation for Statistical Computing, Vienna, Austria). Cohen's kappa coefficient was omitted due to the kappa paradox arising from extreme proportions (0% or 100%).

3. Results

A total of 26 carbapenemase genes representing 12 distinct allelic variants were confirmed by WGS across the 23 isolates (20 single-gene isolates, two isolates co-harboring bla_{VIM} and bla_{IMP}, and one isolate co-harboring bla_{NDM} and $bla_{\text{OXA-48-like}}$). At the allele level, Galaxy Lite detected all 12 distinct carbapenemase alleles evaluated (12/12), whereas both Xpert Carba-R and Molecular Mouse detected 8 alleles (8/12), missing all four IMP variants. Galaxy Lite detected all 26 WGS-confirmed targets evaluated in this study (analytical inclusivity 100%; 95% CI: 86.8–100%). In contrast, Xpert Carba-R detected 14 of 26 targets, yielding an analytical inclusivity of 53.8% (95% CI: 33.4–73.4%). All 12 targets discordant between Galaxy Lite and Xpert Carba-R were IMP genes ($bla_{\text{IMP-16}}$, $n = 2$; $bla_{\text{IMP-18}}$, $n = 3$; $bla_{\text{IMP-74}}$, $n = 2$; $bla_{\text{IMP-93}}$, $n = 5$) (McNemar's exact test at the gene level, $p < 0.001$; isolate-level sensitivity analysis, $p < 0.001$). Molecular Mouse was evaluated on a subset of 13 isolates harboring 16 target genes that included at least one representative of each of the 12 alleles (Table 2); it detected 10 of 16 targets (62.5%; 95% CI: 35.4–84.8%). Because this estimate derives from a different denominator and a different distribution of alleles, it is not directly comparable with the estimates obtained for Galaxy Lite and Xpert Carba-R on the full panel. Within this subset, all six targets discordant with Galaxy Lite were IMP genes (McNemar's exact test at the gene level, $p = 0.031$; isolate-level sensitivity analysis, $p = 0.031$); as with Xpert Carba-R, all false-negative results were restricted to IMP alleles. On the 16 targets tested on both Xpert Carba-R and Molecular Mouse, results were identical (10 concordant positive and 6 concordant negative results; no discordant pairs; Table S2).

This concordance reflects the failure of both assays to detect the same six IMP targets and should not be interpreted as evidence of adequate or equivalent analytical performance. For non-IMP targets (KPC, NDM, OXA-48-like, and VIM), all three platforms detected all tested targets (Galaxy Lite and Xpert Carba-R, 14/14 [95% CI: 76.8–100%]; Molecular Mouse, 10/10 [95% CI: 69.2–100%]) (Table 2). For IMP targets, inclusivity was 100% (12/12; 95% CI: 73.5–100%) for Galaxy Lite, 0% (0/12; 95% CI: 0–26.5%) for Xpert Carba-R, and 0% (0/6; 95% CI: 0–45.9%) for Molecular Mouse.

4. Discussion

In this positive-enriched challenge panel of WGS-characterized Peruvian isolates, all three commercial real-time PCR platforms detected every tested KPC, NDM, OXA-48-like, and VIM target, whereas marked differences were observed for IMP alleles: Xpert Carba-R and Molecular Mouse did not detect any of the tested IMP-16, IMP-18, IMP-74, or IMP-93 targets, whereas Galaxy Lite detected all of them. To our knowledge, this is the first head-to-head comparison of these platforms against the locally predominant IMP-74 and IMP-93 alleles. These findings refer to analytical inclusivity in cultured isolates and should not be extrapolated to clinical sensitivity in patient specimens. Because this challenge panel was purposively constructed with an uneven number of isolates per allele (ranging from one to five targets), overall gene-level inclusivity percentages are weighted by allele representation in the panel. Consequently, allele-level detection profiles (12/12 alleles for Galaxy Lite vs. 8/12 for Xpert Carba-R and Molecular Mouse) provide a more direct assessment of target coverage across circulating variants than aggregate percentages or descriptive *p*-values.

The performance of Xpert Carba-R observed here aligns with previous reports evaluating this platform against regional IMP variants. While Xpert Carba-R exhibits high inclusivity for KPC, NDM, OXA-48-like, and VIM [20,21], reduced detection has been documented for specific IMP alleles, including IMP-18 in Costa Rica [10] and IMP-8, IMP-13, IMP-16, and IMP-18 in Argentina [14]. Notably, in a global challenge set of *P. aeruginosa*, the commercially available Xpert Carba-R detected 58% of IMP-carrying isolates, whereas the research-use-only Xpert Carba-R NxG detected 100% [9]; therefore, the specific version of the Xpert assay should be considered when interpreting IMP detection. Similarly, published evaluations of Molecular Mouse report high sensitivity for common European carbapenemase targets but limited validation data for IMP variants [22,23]. In our panel, Molecular Mouse likewise did not detect the Peruvian IMP alleles tested, although this observation is based on only six IMP targets.

The reasons for the differential detection of IMP alleles were not investigated in this study, because the primer and probe sequences of the commercial assays are proprietary and no in silico or sequencing analysis of their binding regions was performed. Primer/probe–template mismatches are a plausible, but unproven, explanation for the IMP drop-out observed with Xpert Carba-R and Molecular Mouse [7]; this interpretation is consistent with the restoration of IMP detection after primer redesign based on regional IMP sequences in Costa Rica [10]. The IMP family displays substantial genetic diversity [8,24], with sequence identity between subgroups ranging from 80% to 99.6% [25]. We hypothesize that platforms developed in regions with high IMP endemicity and diversity, such as East Asia, may incorporate primer sets designed to cover a broader range of IMP sequences. However, this hypothesis could not be verified because the target regions of the Galaxy Lite assay have not been publicly disclosed. Likewise, whether the assays that failed to detect these alleles were designed using IMP sequences poorly representative of South American alleles remains speculative.

Although clinical outcomes were not assessed in this study, these false-negative results could have relevant clinical and infection-control implications. In Peru, IMP-93 was detected

in 96.3% of ST357 *P. aeruginosa* isolates in a WGS-based study from Lima, in which this high-risk lineage accounted for 38.3% of carbapenemase-producing isolates [5]. A false-negative molecular result could lead clinicians to attribute carbapenem resistance solely to non-enzymatic mechanisms (such as OprD porin loss), potentially resulting in the inappropriate use of newer beta-lactam/beta-lactamase inhibitor combinations (ceftolozane–tazobactam or ceftazidime–avibactam) that are ineffective against MBL producers. Unrecognized MBL producers could also escape contact-precaution protocols, potentially facilitating nosocomial transmission. These potential consequences were not directly evaluated in the present study and warrant confirmation in clinical studies.

Because the inclusivity of real-time PCR assays depends on sequence complementarity at primer and probe binding sites, periodic updates of target formulations by manufacturers, informed by ongoing genomic surveillance, would be desirable, as illustrated by the expanded IMP coverage of the Xpert Carba-R NxG prototype [9]. Our results also support local verification of commercial molecular assays with well-characterized, locally circulating strains before their routine implementation.

This study has several limitations. First, the panel was small (26 targets in 23 isolates) and positively enriched, and individual alleles were represented by only one to five targets; consequently, confidence intervals are wide, and the findings cannot be generalized to other alleles, lineages, or settings. Second, the panel was purposively selected rather than epidemiologically representative, and isolates carrying the same allele may be clonally related (Table S1); thus, individual targets are not fully independent observations. Third, carbapenemase-negative isolates were not included; therefore, analytical specificity and overall diagnostic accuracy could not be assessed. Fourth, Molecular Mouse was tested on only 16 targets, so its overall inclusivity estimate is not directly comparable with those of the other two platforms. Fifth, all assays were performed on pure cultured isolates; performance on direct clinical specimens (e.g., rectal swabs or positive blood cultures), analytical sensitivity (limit of detection), and clinical sensitivity were not evaluated. Sixth, assays were conducted as single runs without repeat testing of unexpected negative results; independent replicate confirmation would further strengthen these findings. Finally, the mechanism underlying IMP target drop-out was not investigated experimentally.

5. Conclusions

In this small, positive-enriched panel of WGS-characterized Peruvian isolates, all three platforms detected all tested KPC, NDM, OXA-48-like, and VIM targets, whereas only Galaxy Lite detected the IMP-16, IMP-18, IMP-74, and IMP-93 targets evaluated. These findings suggest that the allelic diversity of IMP carbapenemases may challenge the design of commercial real-time PCR assays. Local verification with well-characterized circulating strains is advisable before commercial carbapenemase PCR assays are implemented, particularly where IMP alleles are endemic. Future studies should include larger, epidemiologically representative collections with carbapenemase-negative isolates, evaluate direct clinical specimens, and combine in silico or sequencing analyses of primer/probe binding regions to elucidate the mechanisms of IMP target drop-out; updated assay versions should also be re-evaluated against these alleles.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/diagnostics16223870/s1. Table S1: Isolate-level characteristics (species, sequence type, carbapenemase gene[s] and allele[s], WGS accession number) and results obtained with each platform; Table S2: Paired 2 × 2 contingency tables for each pairwise platform comparison at the gene and isolate levels.

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Informed Consent Statement: Patient consent was waived because the study used only archived, de-identified bacterial isolates obtained during routine clinical care.

Data Availability Statement: WGS data are available under the accession numbers listed in Table S1.

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References

1. WHO. *WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance*; World Health Organization: Geneva, Switzerland, 2024.
2. Madney, Y.; Mahfouz, S.; Bayoumi, A.; Hassanain, O.; Hassanain, O.; Sayed, A.A.; Jalal, D.; Lotfi, M.; Tolba, M.; Ziad, G.A.; et al. Carbapenem-Resistant Enterobacteriaceae (CRE) in Children with Cancer: The Impact of Rapid Diagnostics and Targeted Colonization Strategies on Improving Outcomes. *Microorganisms* **2025**, *13*, 1627. [CrossRef] [PubMed]
3. Nordmann, P.; Poirel, L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-Negative Bacteria. *Clin. Infect. Dis.* **2019**, *69*, S521–S528. [CrossRef] [PubMed]
4. Tenover, F.C.; Nicolau, D.P.; Gill, C.M. Carbapenemase-Producing Pseudomonas Aeruginosa –an Emerging Challenge. *Emerg. Microbes Infect.* **2022**, *11*, 811–814. [CrossRef] [PubMed]
5. Tickler, I.A.; La Torre, J.C.G.D.; Alvarado, L.; Obradovich, A.E.; Tenover, F.C. Mechanisms of Carbapenemase-Mediated Resistance among High-Risk Pseudomonas Aeruginosa Lineages in Peru. *J. Glob. Antimicrob. Resist.* **2022**, *31*, 135–140. [CrossRef] [PubMed]
6. Hasan, S.A.; Raoof, W.M.; Ahmed, K.K. First Report Of Co-Harboring Bleomycin Resistance Gene (Blembl) and Carbapenemase Resistance Gene (Blandm-1) Klebsiella Pneumoniae in Iraq With Comparison Study Among the Sensitivity Test, The Bd Phoenix Cpo Detect Test, and The Rapidec® Carba Np Test for Detecting Carbapenem Resistant Gram Negative Bacteria. *Sib. J. Life Sci. Agric.* **2024**, *16*, 208–237. [CrossRef]
7. Stadhouders, R.; Pas, S.D.; Anber, J.; Voermans, J.; Mes, T.H.M.; Schutten, M. The Effect of Primer-Template Mismatches on the Detection and Quantification of Nucleic Acids Using the 5′ Nuclease Assay. *J. Mol. Diagn.* **2010**, *12*, 109–117. [CrossRef] [PubMed]
8. Bonnin, R.A.; Jousset, A.B.; Jacquemin, A.; Emeraud, C.; Girlich, D.; Naas, T.; Dortet, L. Genetic Diversity, Biochemical Properties, Epidemiology, and Detection Methods of IMP- and VIM-Type Carbapenemases. *Clin. Microbiol. Rev.* **2025**, *38*, e00129-23. [CrossRef] [PubMed]
9. Gill, C.M.; Asempa, T.E.; Tickler, I.A.; Cruz, C.D.; Tenover, F.C.; Nicolau, D.P. Evaluation of the Xpert Carba-R NxG Assay for Detection of Carbapenemase Genes in a Global Challenge Set of *Pseudomonas aeruginosa* Isolates. *J. Clin. Microbiol.* **2020**, *58*, e01098-20. [CrossRef] [PubMed]
10. Molina-Mora, J.A.; Rojas-Varela, Á.; Martínez-Arana, C.; Portilla-Victor, L.; Quirós-Fallas, I.; Sánchez-Fonseca, M.; Araya, X.; Cascante-Serrano, D.; Segura-Retana, E.; Espinoza-Solís, C.; et al. Finding the Missing IMP Gene: Overcoming the Imipenemase IMP Gene Drop-Out in Automated Molecular Testing for Carbapenem-Resistant Bacteria Circulating in Latin America. *Antibiotics* **2025**, *14*, 772. [CrossRef] [PubMed]
11. Escalante, E.G.; Champi-Merino, R.G.; Alvarado, L.; Gómez-de-la-Torre, J.C.; Chávez, E.S.; Sandoval-Ahumada, R.; Lazo, G.P.; Janet, C.; Matta-Chuquisapon, M.; Castillo, L.V.M.; et al. Expanding Threat of Carbapenemase-Producing *Escherichia Coli*

and *Klebsiella Pneumoniae* in Peru: Genomic and Phenotypic Evidence of High-Risk Clones Dissemination. *Antimicrob. Agents Chemother.* e00601-26. [CrossRef] [PubMed]
12. Gonzales-Rodriguez, A.; Gómez-de-la-Torre, J.C.; Alvarado, L.; Gonzales Escalante, E. First Report of KPC-35-Producing Klebsiella Pneumoniae ST258 Isolated in Peru. *Rev. Argent. Microbiol.* **2025**, *57*, 3–7. [CrossRef] [PubMed]
13. Ríos, P.; Rocha, C.; Castro, W.; Vidal, M.; Canal, E.; Bernal, M.; Reynolds, N.D.; Tilley, D.H.; Simons, M.P. Extensively Drug-Resistant (XDR) Pseudomonas Aeruginosa Identified in Lima, Peru Co-Expressing a VIM-2 Metallo-β-Lactamase, OXA-1 β-Lactamase and GES-1 Extended-Spectrum β-Lactamase. *JMM Case Rep.* **2018**, *5*, e005154. [CrossRef] [PubMed]
14. Pasteran, F.; De Mendieta, J.; Menocal, M.; Marchetti, P.; Olivieri, L.; Appendino, A.; Pereda, R.; Sangoy, A.; Kuzawka, M.; Tocho, E.; et al. Evaluation of the Ability of the Gene Xpert Carba-R System to Detect KPC and IMP Carbapenemases Variants. In Proceedings of the Abstract Book 2024 34th Congress of the European Society of Clinical Microbiology and Infectious Diseases; ESCMID: Barcelona, Spain; p. 1928.
15. Cuicapuza, D.; Alvarado, L.; Tocasca, N.; Aguilar, D.; Gómez-de-la-Torre, J.C.; Salvatierra, G.; Tsukayama, P.; Tamariz, J. First Report of OXA-181-Producing Enterobacterales Isolates in Latin America. *Microbiol. Spectr.* **2023**, *11*, e0458422. [CrossRef] [PubMed]
16. Krapp, F.; Cuicapuza, D.; Salvatierra, G.; Buteau, J.P.; Amaro, C.; Astocondor, L.; Hinostroza, N.; Jacobs, J.; García, C.; Tsukayama, P. Emerging Carbapenem-Resistant Klebsiella Pneumoniae in a Tertiary Care Hospital in Lima, Peru. *Microbiol. Spectr.* **2025**, *13*, e0182524. [CrossRef] [PubMed]
17. Cuicapuza, D.; Loyola, S.; Velásquez, J.; Fernández, N.; Llanos, C.; Ruiz, J.; Tsukayama, P.; Tamariz, J. Molecular Characterization of Carbapenemase-Producing Enterobacterales in a Tertiary Hospital in Lima, Peru. *Microbiol. Spectr.* **2024**, *12*, e0250323. [CrossRef] [PubMed]
18. Pons, M.J.; Marí-Almirall, M.; Ymaña, B.; Moya-Salazar, J.; Muñoz, L.; Sauñe, S.; Salazar-Hernández, R.; Vila, J.; Roca, I. Spread of St348 Klebsiella Pneumoniae Producing NDM-1 in a Peruvian Hospital. *Microorganisms* **2020**, *8*, 1392. [CrossRef] [PubMed]
19. Lezameta, L.; Cuicapuza, D.; Dávila-Barclay, A.; Torres, S.; Salvatierra, G.; Tsukayama, P.; Tamariz, J. Draft Genome Sequence of a New Delhi Metallo-Lactamase (NDM-1)-Producing Providencia Stuartii Strain Isolated in Lima, Peru. *Microbiol. Resour. Announc.* **2020**, *9*. [CrossRef] [PubMed]
20. Jin, X.; Zhang, H.; Wu, S.; Qin, X.; Jia, P.; Tenover, F.C.; Tang, Y.W.; Li, M.; Hu, F.; Yang, Q.; et al. Multicenter Evaluation of Xpert Carba-R Assay for Detection and Identification of the Carbapenemase Genes in Rectal Swabs and Clinical Isolates. *J. Mol. Diagn.* **2021**, *23*, 111–119. [CrossRef] [PubMed]
21. Li, H.-H.; He, Z.-J.; Xie, L.-M.; Zhang, J.-S.; Xie, T.-A.; Fan, S.-J.; Guo, X.-G. Evaluation of Xpert Carba-R Assay for the Detection of Carbapenemase Genes in Gram-Negative Bacteria. *BioMed Res. Int.* **2021**, *2021*, 6614812. [CrossRef] [PubMed]
22. Ivagnes, V.; De Maio, F.; Baccani, I.; Antonelli, A.; Menchinelli, G.; Rosato, R.; Cafaro, G.; Santarelli, G.; Falletta, F.; D'Inzeo, T.; et al. Detection of β-Lactam Resistance Genes in Gram-Negative Bacteria from Positive Blood Cultures Using a Microchip-Based Molecular Assay. *Front. Cell. Infect. Microbiol.* **2025**, *15*, 1597700. [CrossRef] [PubMed]
23. Chilleri, C.; Salvetti, S.; Coppi, M.; Montenora, I.; Giani, T.; Rossolini, G.M.; Antonelli, A. Evaluation of the Performance of Novel Gram-Negative and Gram-Positive Sepsis Panels for the Rapid Diagnosis of Bloodstream Infections. *Diagnostics* **2026**, *16*, 481. [CrossRef] [PubMed]
24. Li, X.; Mu, X.; Chen, F.; Lu, X.; He, J.; Zheng, Y.; Zhou, D.; Yin, Z.; Wang, P. Characterization of Three Novel IMP Metallo-β-Lactamases, IMP-89, IMP-91, and IMP-96, and Diverse Bla IMP -Carrying Accessory Genetic Elements from Chinese Clinical Isolates. *Microbiol. Spectr.* **2023**, *11*, e04986-22. [CrossRef] [PubMed]
25. Meini, M.R.; Llarrull, L.I.; Vila, A.J. Evolution of Metallo-β-Lactamases: Trends Revealed by Natural Diversity and in Vitro Evolution. *Antibiotics* **2014**, *3*, 285–316. [CrossRef] [PubMed]

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