

Phenotypic and Molecular Profiles of Carbapenem Resistance in Community Infections in the Southern Democratic Republic of Congo

Hichika Tshikolasony¹, Kimuni Kamona², Ilunga Eric Kasamba^{3*} 

¹Higher Institute of Medical Techniques of Kolwezi, Kolwezi, Democratic Republic of Congo

²Higher Institute of Medical Techniques of Lubumbashi, Lubumbashi, Democratic Republic of Congo

³Faculty of Medicine, University Clinics of Lubumbashi, Lubumbashi, Democratic Republic of Congo

Email: *kasambailunga@gmail.com

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Abstract

Background: Carbapenem resistance is an increasing threat, but combined phenotypic and molecular data from community-onset infections in the Democratic Republic of Congo remain scarce. **Methods:** We retrospectively analysed 272 non-duplicate bacterial isolates recovered from urine, pus, and sputum specimens collected between April 2023 and May 2025 in southern Democratic Republic of Congo. Susceptibility to imipenem and meropenem was assessed by disk diffusion and interpreted using EUCAST version 15.0. All 46 imipenem-resistant isolates underwent multiplex PCR for blaNDM, blaOXA-48-like, blaKPC, blaVIM, and blaIMP. Associations were explored using Fisher's exact test and crude odds ratios. **Results:** Urine accounted for 212/272 specimens (77.9%), pus for 40/272 (14.7%), and sputum for 20/272 (7.4%). *Escherichia coli* was the most frequent species (112/272; 41.2%). Resistance was observed in 46/272 isolates for imipenem (16.9%; 95% CI 12.9 - 21.8) and 22/272 for meropenem (8.1%; 95% CI 5.4 - 11.9). Among the 46 isolates tested by PCR, blaNDM was detected in 18 (39.1%), blaOXA-48-like in 12 (26.1%), blaKPC in 7 (15.2%), blaVIM in 5 (10.9%), and blaIMP in 2 (4.3%); no target was detected in 2 isolates (4.3%). An exploratory association was observed between blaVIM and *Pseudomonas aeruginosa* (OR 4.20, 95% CI 1.10 - 16.0; p = 0.03). **Conclusion:** Carbapenem resistance and clinically important carbapenemase genes were present among community-onset isolates. The findings support strengthened phenotypic and molecular surveillance, antimicrobial stewardship, and laboratory capacity.

Keywords

Carbapenem Resistance, Carbapenemase, blaNDM, *Escherichia coli*, *Pseudomonas aeruginosa*, Community-Onset Infection

1. Introduction

Antimicrobial resistance compromises effective treatment and increases the morbidity, mortality, and cost associated with bacterial infections [1] [2]. Carbapenems are often reserved for severe infections caused by multidrug-resistant Gram-negative organisms, particularly extended-spectrum β -lactamase-producing Enterobacterales. Their value is increasingly threatened by resistance mediated by carbapenemases and by non-enzymatic mechanisms such as porin loss and efflux [3]-[6].

Clinically important acquired carbapenemases include New Delhi metallo- β -lactamase (NDM), oxacillinase-48-like enzymes (OXA-48-like), *Klebsiella pneumoniae* carbapenemase (KPC), Verona integron-encoded metallo- β -lactamase (VIM), and imipenemase (IMP). Many are carried on mobile genetic elements that facilitate spread within and between bacterial species [5]-[8]. Although initially emphasized in healthcare-associated infections, carbapenem-resistant organisms are increasingly detected in community-onset infections [6] [9].

Surveillance data remain limited in many sub-Saharan African settings, including southern Democratic Republic of Congo. This study therefore assessed phenotypic resistance to imipenem and meropenem, described selected carbapenemase genes, and explored associations between gene carriage, bacterial species, and specimen type among community-onset isolates.

2. Materials and Methods

Study design, setting, and data source

This retrospective cross-sectional analytical study used microbiology and molecular-testing records for specimens processed between April 2023 and May 2025 in southern Democratic Republic of Congo.

The names and roles of the participating laboratories and the exact source registers should be inserted here before submission.

Participants and isolate selection

Eligible records were from patients evaluated for a presumed community-onset urinary, wound/soft-tissue, or respiratory infection and whose urine, pus, or sputum specimen yielded a clinically significant bacterial isolate with interpretable imipenem and meropenem susceptibility results. A community-onset infection was defined as an infection diagnosed in an outpatient or within the first 48 hours after admission, without hospitalization, surgery, dialysis, residence in a long-term care facility, or parenteral antibiotic therapy during the preceding 90 days.

Records were excluded when the specimen source or patient identifier was missing, bacterial identification was unreliable, susceptibility results were incomplete or uninterpretable, the culture was considered contaminated, a stored isolate was non-viable, or the available history was compatible with a healthcare-associated infection. Isolates for which carbapenem interpretation was intrinsically inappropriate were excluded from the corresponding carbapenem-resistance analysis.

To prevent over-representation, only the first isolate of a given species from the same patient, specimen type, and infection episode was retained. A further isolate of the same species recovered from the same patient within 30 days was considered a duplicate unless it originated from a different infection site or had a clearly distinct phenotypic resistance profile.

Culture and bacterial identification

Specimens were inoculated on MacConkey agar, mannitol-salt agar, and blood agar as appropriate and incubated aerobically at 35 - 37°C for 18 - 24 hours. Isolates were identified using colony morphology, Gram staining, oxidase, catalase and coagulase tests, and API 20E for Enterobacterales. Isolates selected for molecular analysis were stored in glycerol broth at -20°C or -80°C, according to local availability, and subcultured before DNA extraction.

Antimicrobial susceptibility testing

We performed disk diffusion on Mueller-Hinton agar using 10-µg imipenem and 10-µg meropenem disks. All available zone diameters were interpreted retrospectively using the European Committee on Antimicrobial Susceptibility Testing clinical breakpoint tables, version 15.0 (2025) [10]. EUCAST categories were S (susceptible, standard dosing regimen), I (susceptible, increased exposure), and R (resistant); only category R was counted as resistant in prevalence estimates. Carbapenem analysis was restricted to Gram-negative species for which EUCAST provides relevant interpretive criteria. Carbapenem results reported for *S. aureus* were reviewed separately and were not used to infer carbapenemase-mediated resistance.

Quality control was performed with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. A run was accepted only when control-zone diameters were within the EUCAST quality-control ranges; out-of-range results triggered investigation and repetition of the run.

PCR detection of carbapenemase genes

We selected all 46 isolates classified as resistant to imipenem for molecular testing after recovery from storage; no additional sampling was performed within this group. Isolates had to remain viable after storage, have confirmed identification, and yield DNA of sufficient quality. A multiplex PCR based on the protocol of Poirel *et al.* [11] targeted blaNDM, blaOXA-48-like, blaKPC, blaVIM, and blaIMP (as shown in Table 1).

Each 25-µL reaction contained 12.5 µL of 2× PCR master mix, each primer at a final concentration of approximately 0.2 µM, 2 µL of extracted DNA, and nucle-

ase-free water. Amplification comprised initial denaturation at 94°C for 10 min; 36 cycles of 94°C for 30 s, 52°C for 40 s, and 72°C for 50 s; and final extension at 72°C for 5 min. Amplicons were separated on 1.5% agarose gel with a 100-bp DNA ladder and visualized under ultraviolet or blue light after staining.

Table 1. Primer sequences used for PCR amplification of carbapenemase-encoding genes.

Target	Forward primer 5'–3'	Reverse primer 5'–3'	Amplicon	Reference/control
blaNDM	GGTTTGGCGATCTGGTTTTTC	CGGAATGGCTCATCACGATC	621 bp	[11] [12]
blaOXA-48-like	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438 bp	[11]
blaKPC	CGTCTAGTTCTGCTGTCTTG	CTTGTCATCCTTGTTAGGCG	798 bp	[11]
blaVIM	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG	390 bp	[11]
blaIMP	GGAATAGAGTGGCTTAAYTCTC	GGTTTAAYAAAACAACCACC	232 bp	[11]

Each run included gene-specific positive controls consisting of DNA from previously characterized isolates or reference material, an extraction-negative control, and a no-template control containing nuclease-free water. A run was valid only when the expected positive-control bands were present and both negative controls showed no amplification. The multiplex design allowed detection of more than one gene in an isolate. Each target was coded independently as present or absent; multiple bands of the expected sizes were interpreted as co-detection. In the present dataset, the reported mutually exclusive categories indicate that no co-detection was observed.

The authors verified the approved methodological description against the original specimen-reception registers, bacterial-identification records, antimicrobial-susceptibility result sheets, strain-storage records, PCR worksheets, thermocycler records, and gel-electrophoresis images. The study period, specimen counts, isolate identification, phenotypic resistance results, selection of isolates for molecular testing, PCR target results, and absence of recorded gene co-detection were concordant with the source documents. Duplicate isolates were checked using the patient identifier, bacterial species, specimen type, and collection date. Any discrepancy identified during verification was resolved by reference to the original dated laboratory record before the analytical database was finalized.

Statistical analysis

Categorical variables were summarized as counts and percentages. Resistance prevalence was reported with Wilson 95% confidence intervals. Species-gene associations were evaluated using Fisher's exact test, with crude odds ratios (ORs) and 95% confidence intervals. Two-sided *p* values below 0.05 were considered statistically significant. A conventional multivariable logistic-regression model was not retained because 44 of the 46 PCR-tested isolates carried at least one target and only two were gene-negative. This extreme outcome imbalance would provide insufficient information for a stable model with multiple covariates and could produce complete or quasi-complete separation. Species and specimen type were

therefore treated as exploratory variables in bivariate analyses. “Other Gram-negative bacteria” served as the species reference category and urine as the specimen reference category when odds ratios were estimable.

Ethics

The study protocol was reviewed and approved by the Medical Ethics Committee of the University of Lubumbashi, Democratic Republic of Congo (approval number: [INSERT OFFICIAL NUMBER]; approval date: [INSERT DATE]). Because this retrospective study used existing anonymized microbiological records, the requirement for individual informed consent was waived by the ethics committee. Patient confidentiality was maintained throughout the study, and no directly identifying information was included in the analytical database.

The approval number, approval date, and written confirmation of the consent waiver must be copied exactly from the official ethics decision before submission.

3. Results

Specimens and bacterial isolates

A total of 272 non-duplicate isolates were included. Urine contributed 212 isolates (77.9%), pus 40 (14.7%), and sputum 20 (7.4%) (as shown in **Figure 1**). Thus, all three specimen categories account for the full denominator. *E. coli* was the most frequent species (112/272; 41.2%), followed by *S. aureus* (33/272; 12.1%).

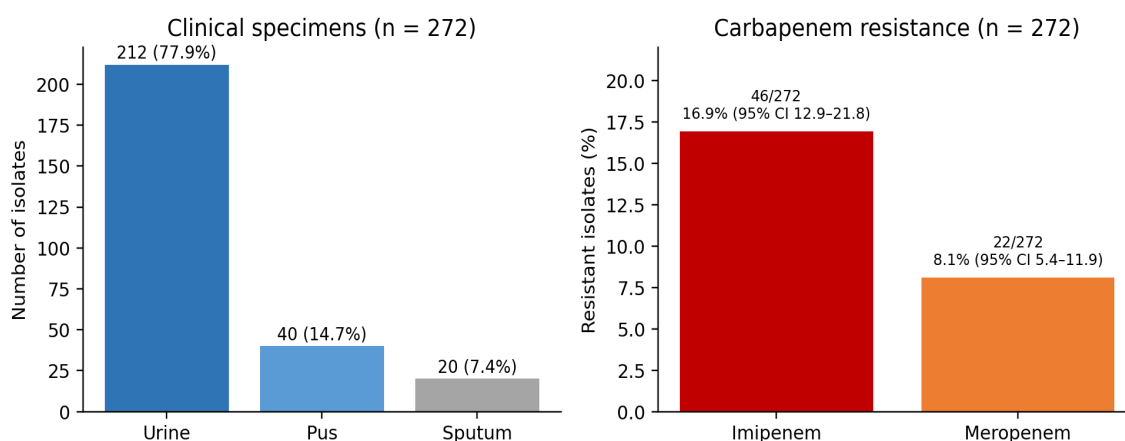


Figure 1. Distribution of specimens and overall phenotypic resistance. Confidence intervals are Wilson 95% confidence intervals.

Phenotypic carbapenem resistance

Imipenem resistance was identified in 46/272 isolates in the overall study cohort (16.9%; 95% CI 12.9 - 21.8), whereas meropenem resistance was identified in 22/272 (8.1%; 95% CI 5.4 - 11.9). The 33 *Staphylococcus aureus* isolates were excluded from species-specific carbapenem analysis. Among the 239 eligible Gram-negative isolates, the corresponding resistance proportions were 46/239 (19.2%) for imipenem and 22/239 (9.2%) for meropenem. Overall resistance estimates are presented in **Table 2**, and species-specific results are presented in **Table 3**.

Table 2. Prevalence of phenotypic resistance to carbapenems among bacterial isolates (N = 272).

Drug	Resistant n/N	Resistance %	95% CI
Imipenem	46/272	16.9	12.9 - 21.8
Meropenem	22/272	8.1	5.4 - 11.9

Species-specific resistance was calculated using the number of eligible Gram-negative isolates of each species as the denominator. Among 112 *Escherichia coli* isolates, 9 were resistant to imipenem (8.0%) and 8 to meropenem (7.1%). Among 34 *Pseudomonas aeruginosa* isolates, 8 were resistant to imipenem (23.5%) and 5 to meropenem (14.7%). Among 19 *Citrobacter freundii* isolates, 4 were resistant to imipenem (21.1%) and 3 to meropenem (15.8%). The remaining 74 Gram-negative isolates included 25 imipenem-resistant isolates (33.8%) and 6 meropenem-resistant isolates (8.1%). These counts reproduce the aggregate totals of 46 and 22 resistant isolates, respectively (as shown in **Table 3**).

Table 3. Species-specific resistance to imipenem and meropenem among eligible Gram-negative isolates.

Bacterial species	Tested N	Imipenem resistant n (%)	Meropenem resistant n (%)	Analytical status
<i>Escherichia coli</i>	112	9 (8.0)	8 (7.1)	Eligible Gram-negative isolates
<i>Pseudomonas aeruginosa</i>	34	8 (23.5)	5 (14.7)	Eligible Gram-negative isolates
<i>Citrobacter freundii</i>	19	4 (21.1)	3 (15.8)	Eligible Gram-negative isolates
Other Gram-negative species	74	25 (33.8)	6 (8.1)	Eligible Gram-negative isolates
Subtotal: Gram-negative isolates	239	46 (19.2)	22 (9.2)	Included in species-specific analysis
<i>Staphylococcus aureus</i>	33	Not applicable	Not applicable	Excluded from carbapenem analysis
Total study isolates	272	46 (16.9)	22 (8.1)	Overall study cohort

Carbapenemase genes among PCR-tested isolates

PCR was performed on 46 imipenem-resistant isolates. blaNDM was detected in 18/46 (39.1%), blaOXA-48 in 12/46 (26.1%), blaKPC in 7/46 (15.2%), blaVIM in 5/46 (10.9%), and blaIMP in 2/46 (4.3%); none of the five targets was detected in 2/46 isolates (4.3%) (as shown in **Table 4**).

Table 4. Distribution of carbapenemase genes among imipenem-resistant bacterial isolates (N = 46).

PCR result	n/N	%	95% CI
blaNDM	18/46	39.1	26.4 - 53.5
blaOXA-48	12/46	26.1	15.6 - 40.3
blaKPC	7/46	15.2	7.6 - 28.2
blaVIM	5/46	10.9	4.7 - 23.0
blaIMP	2/46	4.3	1.2 - 14.5
No target detected	2/46	4.3	1.2 - 14.5

Although the multiplex assay could detect several targets in one isolate, the reported molecular categories were mutually exclusive, and no co-detection was observed.

Bivariate associations

The only reported statistically significant species-gene association was between *P. aeruginosa* and blaVIM (OR 4.20, 95% CI 1.10 - 16.0; $p = 0.03$). All other reported intervals included 1. Because several species-gene comparisons were performed, these exploratory results should be interpreted cautiously (as shown in Table 5).

Table 5. Associations between bacterial species and carbapenemase genes.

Species	Gene	OR	95% CI	p
<i>E. coli</i>	blaNDM	1.91	0.62 - 5.82	0.25
<i>E. coli</i>	blaOXA-48	2.40	0.70 - 8.10	0.16
<i>E. coli</i>	blaKPC	0.55	0.09 - 3.10	0.50
<i>P. aeruginosa</i>	blaNDM	2.75	0.80 - 9.40	0.11
<i>P. aeruginosa</i>	blaVIM	4.20	1.10 - 16.0	0.03
<i>P. aeruginosa</i>	blaOXA-48	0.60	0.12 - 2.90	0.52
<i>C. freundii</i>	blaOXA-48	3.10	0.85 - 11.2	0.08
<i>C. freundii</i>	blaNDM	1.20	0.25 - 5.60	0.80

Choice of regression strategy

Because 44 of 46 PCR-tested isolates were positive for at least one target, a standard multivariable logistic model was not considered reliable. The previously reported adjusted estimates were therefore removed. The association between *P. aeruginosa* and blaVIM is presented as an exploratory bivariate finding and not as evidence of an independent risk factor.

4. Discussion

This study documents phenotypic carbapenem resistance and five clinically important carbapenemase targets among community-onset isolates in the southern Democratic Republic of Congo. Two observations merit emphasis: resistance to imipenem was more frequent than resistance to meropenem, and blaNDM was the most frequently detected target among the 46 PCR-tested isolates. The observed association between *P. aeruginosa* and blaVIM is exploratory and should not be interpreted as an independent causal relationship.

The predominance of urine specimens and *E. coli* is consistent with the large contribution of urinary tract infection to community microbiology workloads. However, specimen composition should not be interpreted as disease incidence because the study denominator comprised submitted culture-positive specimens rather than a population-based sample. The corrected specimen distribution in-

cludes sputum (20/272; 7.4%), which was omitted from the original **Figure 1**.

The overall resistance estimates were 16.9% for imipenem and 8.1% for meropenem. Differences between the two drugs may reflect organism composition, test variability, interpretive criteria, or underlying resistance mechanisms. Direct biological interpretation requires confirmation that one breakpoint standard was applied consistently, reconciliation of species-specific resistant counts with the aggregate totals, and review of the unexpected reporting of carbapenem susceptibility for *S. aureus*.

Among isolates selected for PCR, blaNDM and blaOXA-48-like were the most frequent targets. Both enzyme families have disseminated widely through mobile genetic elements and are important in Enterobacterales [5]-[9]. blaVIM is well recognized in *P. aeruginosa*, making the observed bivariate association biologically plausible [13] [14]. Nevertheless, the wide confidence interval indicates limited precision. Because only two PCR-tested isolates were negative for all five targets, the data did not support a conventional multivariable model.

The finding that some phenotypically resistant isolates lacked all five targets is expected because resistance may involve untested carbapenemases or non-enzymatic mechanisms. Conversely, targeted PCR establishes the presence of a gene but not its expression, genomic context, or clonal relatedness. Sequencing and expanded phenotypic confirmation would strengthen future surveillance.

Strengths and limitations

The study combines phenotypic and molecular observations from a setting with limited published surveillance. Its limitations include retrospective data quality, incomplete clinical variables, a geographically restricted sampling frame, possible selection bias in specimen submission, limited molecular sample size, targeted detection of only five genes, absence of sequencing, and unresolved discrepancies between species-specific resistant counts and aggregate resistance totals. Community-onset status may have been misclassified when prior healthcare exposure was incompletely recorded. The extreme imbalance between gene-positive and gene-negative isolates precluded a reliable conventional multivariable model.

5. Conclusion

Carbapenem resistance was detected among community-onset bacterial isolates in southern Democratic Republic of Congo, with higher overall resistance to imipenem than to meropenem. Among the 46 imipenem-resistant isolates tested by targeted PCR, blaNDM was most frequent, and blaVIM was associated with *P. aeruginosa*. These findings support standardized susceptibility testing, molecular surveillance, antimicrobial stewardship, and confirmatory multicentre studies with complete clinical data and genomic characterization.

Declarations

Ethics Approval: Medical Ethics Committee of the University of Lubumbashi, Democratic Republic of Congo; approval number CE2156/25/UNILU; approval

date 21-05-2025. The requirement for individual informed consent was waived because the study was retrospective and used anonymized records.

Consent for Publication: Not applicable. This manuscript does not contain any identifiable individual-level information, images, or personal data requiring consent for publication.

Data Availability: The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request, subject to approval by the relevant institutional and ethics authorities and compliance with applicable confidentiality requirements.

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Author Contributions

Hichika Tshikolasony contributed to conceptualization, investigation, data collection, data curation, and preparation of the original draft. Kimuni B. Kamona contributed to methodology, laboratory investigation, validation, and critical review of the manuscript. Kasamba Ilunga Eric contributed to conceptualization, methodology, supervision, formal analysis, validation, project administration, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the integrity of the work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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