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Phenotypic Characterisation of Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates and In Vitro Antibacterial Activity of Cinnamomum verum Bark Essential Oil at a Tertiary Care Centre

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ABSTRACT

Background: Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is an important healthcare-associated pathogen, and carbapenem resistance further limits available treatment options. Increasing antimicrobial resistance has also encouraged investigation of plant-derived products such as Cinnamomum verum for potential antibacterial activity. Objective: To characterise MDR *P. aeruginosa* isolates phenotypically, determine carbapenem resistance and carbapenemase-associated phenotypes, and evaluate the in vitro antibacterial activity of *C. verum* bark extract.

Methods: This hospital-based descriptive observational study included 132 non-duplicate MDR *P. aeruginosa* isolates recovered from clinical specimens over two years at a tertiary-care centre in Indore, India. Identification was performed using standard microbiological methods, and antimicrobial susceptibility was determined by the Kirby–Bauer disc-diffusion method according to CLSI M100, 34th edition (2024). Carbapenem-resistant isolates were evaluated using the imipenem-EDTA double-disc synergy test (DDST), Modified Hodge Test (MHT), Carba NP test, modified carbapenem inactivation method (mCIM), and EDTA-modified carbapenem inactivation method (eCIM). The antibacterial activity of *C. verum* bark extract was assessed by disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

Results: Of the 132 MDR isolates, 53 (40.2%; 95% confidence interval: 32.2%–48.7%) were carbapenem resistant. Among these, 48 (90.6%) were DDST positive, 32 (60.4%) MHT positive, 53 (100%) Carba NP positive, and 29 (54.7%) mCIM positive. Of the 29 mCIM-positive isolates, 18 (62.1%) were eCIM positive. Based on the study-defined zone-diameter categories, *C. verum* was very active against 122 of 132 MDR isolates (92.4%) and active against the remaining 10 (7.6%). Among carbapenem-resistant isolates, 49 (92.5%) were classified as very active and four (7.5%) as active. MIC values were predominantly 0.5–2 mg/mL, while MBC values were mainly 1–4 mg/mL.

Conclusion: Carbapenem resistance was common among the MDR *P. aeruginosa* isolates studied. The phenotypic assays produced different positivity rates and

require method-specific interpretation and molecular confirmation. *C. verum* bark extract demonstrated promising in vitro antibacterial activity; however, chemical standardisation, toxicity assessment, molecular studies, and in vivo and clinical evaluation are required before therapeutic application.

Keywords: *Pseudomonas aeruginosa*; multidrug resistance; carbapenem resistance; carbapenemase; *Cinnamomum verum*; antibacterial activity.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic, non-fermenting Gram-negative bacillus that can survive in a wide range of environments. As an opportunistic pathogen, it is particularly important in critically ill and immunocompromised patients and in those with indwelling medical devices. It commonly causes pneumonia, bloodstream infections, urinary tract infections, and wound infections. Its clinical success is supported by intrinsic and acquired resistance mechanisms, rapid adaptive responses, and a broad range of virulence factors [1-3].

MDR *P. aeruginosa* is generally defined as an isolate that is non-susceptible to at least one agent in three or more clinically relevant antimicrobial categories [4]. The organism can resist treatment through several pathways, including reduced outer-membrane permeability, loss or alteration of OprD, increased expression of multidrug efflux pumps, target modification, derepression of chromosomal AmpC, and acquisition of extended-spectrum beta-lactamases or carbapenemases [1,2,5]. Carbapenem resistance is especially concerning because it may result either from carbapenemase production or from a combination of other resistance mechanisms. Phenotypic confirmation therefore remains valuable for surveillance and infection-control activities [5,6].

The available phenotypic assays detect different aspects of carbapenem resistance and may therefore produce different results. The imipenem-EDTA DDST screens for metallo-beta-lactamase activity, Carba NP identifies imipenem hydrolysis, mCIM demonstrates carbapenem inactivation, and eCIM helps differentiate metallo-beta-lactamase activity among mCIM-positive isolates [7-11]. Because no single phenotypic test identifies every resistance mechanism, the results must be interpreted with care, and molecular testing is still required for definitive gene-level characterisation.

The growing burden of antimicrobial resistance has renewed interest in plant-derived compounds with antibacterial and antibiofilm properties. *Cinnamomum verum* contains several bioactive constituents, particularly cinnamaldehyde, that have shown in vitro activity against *P. aeruginosa* [12-15]. However, the observed activity can vary considerably with the plant part, extraction procedure, chemical composition, solvent, inoculum, and test conditions. Laboratory activity alone does not establish clinical effectiveness, and subinhibitory exposure may even trigger adaptive responses, including increased efflux-pump expression [16].

Against this background, the present study characterised MDR *P. aeruginosa* isolates recovered at a tertiary-care centre, compared the positivity of several carbapenemase-associated phenotypic tests among carbapenem-resistant isolates, and evaluated the in vitro antimicrobial activity of *C. verum*.

The study aimed to phenotypically characterise clinical *Pseudomonas aeruginosa* isolates, determine their antimicrobial susceptibility patterns and multidrug-resistance prevalence, and evaluate the in vitro antibacterial activity of *Cinnamomum verum* bark essential oil against MDR isolates.

MATERIALS AND METHODS

Study design and setting

A prospective laboratory-based study was carried out over two years in the Department of Microbiology, Index Medical College Hospital and Research Centre, Malwanchal University, Indore, Madhya Pradesh, India.

Sample size

A total of 132 consecutive, non-duplicate clinical isolates of MDR *P. aeruginosa* obtained during the study period were included. Only the first isolate recovered from each patient during the same infection episode was considered.

Inclusion criteria

- Clinical isolates identified as *P. aeruginosa*.

- Isolates obtained from specimens such as urine, pus, wound swabs, respiratory samples, blood, body fluids, or other clinically relevant specimens.
- The first isolate of *P. aeruginosa* obtained from each patient during the defined infection episode.
- Isolates for which complete antimicrobial susceptibility results were available.

Exclusion criteria

- Duplicate isolates from the same patient and the same infection episode.
- Mixed cultures in which *P. aeruginosa* could not be reliably isolated.
- Non-viable, contaminated, or improperly preserved isolates.
- Isolates with incomplete identification or susceptibility results.

Specimen collection and processing

Clinical specimens were collected using aseptic precautions and transported promptly to the microbiology laboratory. Samples were inoculated onto appropriate culture media, including blood agar, MacConkey agar, cetrimide agar and CLED agar. Plates were incubated aerobically at 35–37°C for 18–24 hours and examined for bacterial growth.

Identification of *Pseudomonas aeruginosa*

Depending on the specimen type, samples were inoculated onto blood agar, MacConkey agar, cetrimide agar, or cystine-lactose-electrolyte-deficient agar and incubated aerobically at 35–37°C for 18–24 hours. *P. aeruginosa* was identified from its colony morphology, pigment production, Gram-stain appearance, oxidase reaction, motility, oxidative utilisation of glucose, and standard biochemical reactions. *P. aeruginosa* ATCC 27853 served as the quality-control strain.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by the Kirby-Bauer disc-diffusion method on Mueller-Hinton agar using a 0.5 McFarland inoculum. After incubation at 35 ± 2°C for 16–18 hours, inhibition-zone diameters were interpreted according to CLSI M100, 34th edition (2024) [6]. The antimicrobial panel was grouped as follows: piperacillin–tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), and amikacin (30 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg).

Definition of multidrug resistance

MDR classification is based on antimicrobial susceptibility testing (AST), not on DDST, MHT, Carba NP, mCIM, or eCIM. These phenotypic tests are performed subsequently on carbapenem-resistant isolates to characterize carbapenemase-associated activity.

MDR was defined as non-susceptibility to at least one drug in three or more relevant antimicrobial categories [4]. Isolates non-susceptible to imipenem, meropenem, and/or doripenem were categorised as carbapenem-resistant *P. aeruginosa* (CRPA).

Phenotypic detection of carbapenemase-associated activity among carbapenem-resistant *Pseudomonas aeruginosa*

All 53 carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates were evaluated for carbapenemase-associated activity using the imipenem–EDTA double-disc synergy test (DDST), Modified Hodge Test (MHT), Carba NP test, and modified carbapenem inactivation method (mCIM). [7–10]. The EDTA-modified carbapenem inactivation method (eCIM) was performed only for mCIM-positive isolates to differentiate an EDTA-inhibitable metallo-β-lactamase (MBL) phenotype. MDR was determined by antimicrobial susceptibility testing and was defined as non-susceptibility to at least one antimicrobial agent in three or more relevant antimicrobial categories; the phenotypic assays were used subsequently to characterize carbapenemase-associated activity among CRPA isolates. [6,11]

Preparation and in vitro testing of *Cinnamomum verum*

Cinnamomum verum bark was shade-dried, powdered, extracted with 80% ethanol, and reconstituted in dimethyl sulfoxide (DMSO). Antibacterial activity was evaluated in triplicate by disc diffusion using 6-mm extract-impregnated discs, with DMSO as the negative control. Mean inhibition zones were categorised as inactive (<9 mm), partially active (9–12 mm), active (13–18 mm), or very active (>18 mm); these study-defined categories were not CLSI clinical breakpoints. MIC was determined by broth microdilution, MBC by subculture from wells showing no visible growth, and biofilm biomass by the microtitre-plate crystal-violet assay [17]. Botanical authentication, extraction ratio and yield, extract concentration, and MIC/MBC dilution range should be reported from the original laboratory records.

Statistical analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS statistics version no. 29. Categorical variables were expressed as frequencies and percentages. Associations between Categorical variables variables were analysed using Chi-square test or Fisher's exact test as appropriate. A p-value <0.05 was considered statistically significant.

Ethical approval

The study received clearance from the Institutional Ethics Committee of Index Medical College Hospital and Research Center, affiliated with Malwanchal University, under approval number MU/Research/EC/Ph.D./2023/300. Patient confidentiality was preserved during the study. Informed consent was obtained from participants, or a waiver was granted by the ethics committee, if relevant.

RESULTS

Study-isolate profile

All 132 non-duplicate MDR *P. aeruginosa* isolates that met the eligibility criteria were included in the final analysis. The source dataset did not record the total number of specimens processed, all *P. aeruginosa* isolates recovered, or the number excluded at each screening stage; therefore, the overall isolation rate and MDR prevalence could not be determined. Of the included isolates, 81 (61.4%) were from male patients and 51 (38.6%) from female patients. Patients older than 60 years formed the largest age group (36/132, 27.3%), followed by those aged 31-45 years (35/132, 26.5%). Table 1 presents the complete distribution by age and sex.

Table 1. Age- and sex-wise distribution of MDR *Pseudomonas aeruginosa* isolates (n=132)

Age group (years)	Male, n	Female, n	Total, n (%)
<=18	9	7	16 (12.1)
19-30	11	7	18 (13.6)
31-45	18	17	35 (26.5)
46-60	19	8	27 (20.5)
>60	24	12	36 (27.3)
Total	81	51	132 (100.0)

Department-wise distribution

Surgery contributed the largest number of isolates (27/132, 20.5%), followed by ENT (15/132, 11.4%). Orthopaedics, Pulmonary Medicine, and Urology each contributed 13 isolates (9.8%). Overall, these five departments accounted for 81 of the 132 isolates (61.4%) (Table 2).

Table 2. Department-wise distribution of MDR *Pseudomonas aeruginosa* isolates

Department	Isolates, n (%)
Surgery	27 (20.5)
ENT	15 (11.4)
Orthopaedics	13 (9.8)
Pulmonary Medicine	13 (9.8)
General Medicine	12 (9.1)
Urology	13 (9.8)
Emergency Medicine	9 (6.8)
Gynaecology	8 (6.1)
Paediatrics	8 (6.1)
PICU	3 (2.3)
NICU	2 (1.5)
Ophthalmology	2 (1.5)
Nephrology	2 (1.5)
MICU	2 (1.5)
Skin and VD	1 (0.8)
SICU	1 (0.8)
Neurology	1 (0.8)
Total	132 (100.0)

Specimen-wise distribution

Pus was the most common specimen, accounting for 36 isolates (27.3%), followed closely by urine with 34 (25.8%). Sputum yielded 18 isolates (13.6%), other swabs 16 (12.1%), and blood 13 (9.8%). Together, pus and urine contributed 70 of the 132 isolates (53.0%) (Table 3).

Table 3. Specimen-wise distribution of MDR *Pseudomonas aeruginosa* isolates (n=132)

Specimen	Isolates, n (%)
Pus	36 (27.3)
Urine	34 (25.8)
Sputum	18 (13.6)
Swab	16 (12.1)
Blood	13 (9.8)
Endotracheal aspirate	5 (3.8)
Tracheostomy aspirate	3 (2.3)
Tracheal specimen	2 (1.5)
Stool	1 (0.8)
Cerebrospinal fluid	1 (0.8)
Ascitic fluid	1 (0.8)
Vaginal discharge	1 (0.8)
Bronchoalveolar lavage fluid	1 (0.8)
Total	132 (100.0)

Antimicrobial susceptibility pattern

Among the 132 *Pseudomonas aeruginosa* isolates, the highest susceptibility was observed to amikacin (56.8%), followed by piperacillin–tazobactam (53.8%) and ceftazidime (51.5%). In contrast, high resistance was noted to meropenem (78.8%) and imipenem (76.5%). Aztreonam and fluoroquinolones also showed considerable resistance. Overall, the isolates demonstrated substantial resistance to several commonly used antipseudomonal agents.

Table 4. Antimicrobial susceptibility profile of MDR *Pseudomonas aeruginosa* isolates (n = 132)

Antimicrobial agent	Susceptible, n (%)	Resistant, n (%)
Piperacillin–tazobactam	71 (53.8)	61 (46.2)
Ceftazidime	68 (51.5)	64 (48.5)
Cefepime	65 (49.2)	67 (50.8)
Aztreonam	52 (39.4)	80 (60.6)
Amikacin	75 (56.8)	57 (43.2)
Ciprofloxacin	59 (44.7)	73 (55.3)
Levofloxacin	55 (41.7)	77 (58.3)
Imipenem	31 (23.5)	101 (76.5)
Meropenem	28 (21.2)	104 (78.8)

Carbapenem resistance

Of the 132 MDR isolates, 53 were classified as CRPA, giving a within-cohort proportion of 40.2% (95% confidence interval, 32.2%–48.7%). The remaining 79 isolates (59.8%) were susceptible to the antipseudomonal carbapenems tested (Table 5). In practical terms, about two in every five isolates in this preselected MDR cohort were carbapenem resistant. This figure describes the study cohort and should not be interpreted as the hospital-wide prevalence of CRPA.

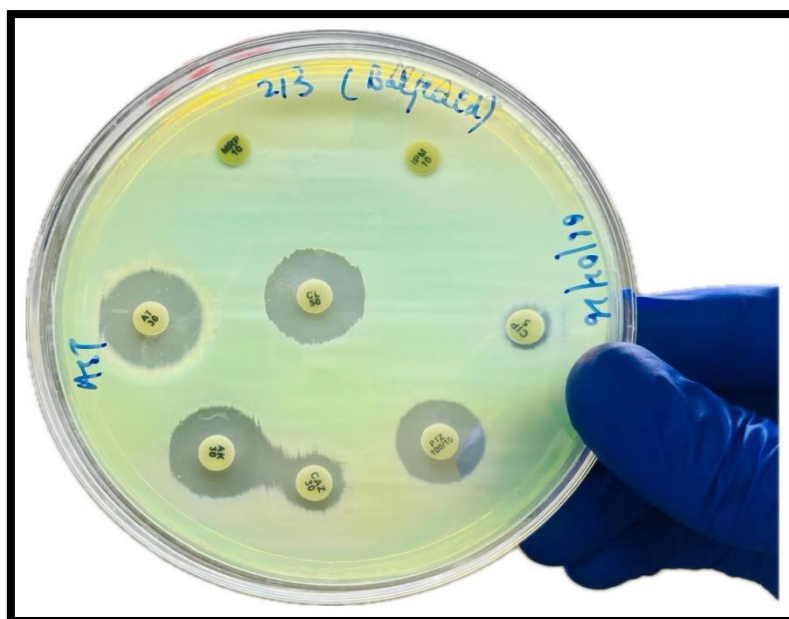


Figure 1

Table 5. Carbapenem-resistance status among MDR *Pseudomonas aeruginosa* isolates

Resistance category	Isolates, n (%)
Carbapenem-resistant MDR <i>P. aeruginosa</i>	53 (40.2)
Carbapenem-susceptible MDR <i>P. aeruginosa</i>	79 (59.8)
Total	132 (100.0)

Phenotypic carbapenemase-associated tests

Positivity varied considerably across the five phenotypic assays. Carba NP was positive in all 53 CRPA isolates (100.0%), followed by DDST in 48 (90.6%), MHT in 32 (60.4%), and mCIM in 29 (54.7%). eCIM was interpreted only for the 29 mCIM-positive isolates; 18 (62.1%) showed an EDTA-inhibitable MBL phenotype, while 11 (37.9%) did not (Table 6). Because eCIM was performed only in the mCIM-positive subgroup, its positivity rate should not be directly ranked against tests performed in all 53 CRPA isolates.

INTERPRETATION: Among tests performed on all 53 CRPA isolates, positivity ranked Carba NP (100%) > DDST (90.6%) > MHT (60.4%) > mCIM (54.7%). eCIM (62.1%) was performed only among the 29 mCIM-positive isolates and therefore should be reported separately, not ranked directly with the four tests above.

Table 6. Positivity of phenotypic carbapenemase-associated tests among CRPA isolates

Test	Eligible isolates, n	Positive, n (%)	Negative, n (%)
DDST	53	48 (90.6)	5 (9.4)
Carba NP	53	53 (100.0)	0 (0.0)
MHT	53	32 (60.4)	21 (39.6)
mCIM	53	29 (54.7)	24 (45.3)
eCIM among mCIM-positive isolates	29	18 (62.1)	11 (37.9)

In vitro antibacterial activity of *Cinnamomum verum*

According to the study-defined zone-diameter categories, *C. verum* bark extract was very active against 122 of the 132 MDR isolates (92.4%) and active against the remaining 10 (7.6%). None of the isolates fell into the partially active or inactive categories. Within the 53-isolate CRPA subgroup, 49 (92.5%) were classified as very active and four (7.5%) as active (Table 6), a distribution similar to that observed in the full MDR cohort. Reported MIC values were predominantly 0.5-2 mg/mL, while MBC values were mainly 1-4 mg/mL. These values are experimental endpoints. Because validated

clinical breakpoints for *C. verum* are not available, terms such as susceptible, intermediate, and resistant should not be applied.

Table 7. In vitro disc-diffusion activity of *Cinnamomum verum* bark extract

Isolate group	Very active, n (%)	Active, n (%)	Partially active/inactive, n (%)
All MDR isolates (n=132)	122 (92.4)	10 (7.6)	0 (0.0)
CRPA isolates (n=53)	49 (92.5)	4 (7.5)	0 (0.0)



Figure 2

DISCUSSION

The present study examined 132 multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates and found that 53 (40.2%) were resistant to at least one antipseudomonal carbapenem. This proportion indicates a substantial burden of carbapenem resistance within the selected MDR population. Mohanty et al. reported carbapenem resistance in 40% of *P. aeruginosa* isolates, which closely agrees with the present finding [18]. In contrast, Verma et al. identified carbapenem resistance in 18.2% of 559 consecutive *P. aeruginosa* isolates from a tertiary-care hospital in eastern India [19]. The higher proportion in the present study is expected because only isolates already classified as MDR were enrolled, whereas Verma et al. analysed a broader consecutive isolate population. Differences in case mix, specimen distribution, antimicrobial exposure, local prescribing practices, infection-control measures, and resistance definitions may also contribute to the variation between centres.

The predominance of isolates from surgical services and the frequent recovery of *P. aeruginosa* from pus and urine are clinically plausible, given the organism's association with wound, device-associated, and urinary infections. However, the present dataset did not include all specimens processed or all *P. aeruginosa* isolates recovered during the study period. The departmental and specimen distributions should therefore be interpreted as the composition of this MDR cohort rather than as department-specific prevalence or infection rates.

The phenotypic assays produced markedly different positivity rates: Carba NP was positive in all 53 CRPA isolates (100%), DDST in 48 (90.6%), MHT in 32 (60.4%), and mCIM in 29 (54.7%). These figures represent phenotypic positivity rates and should not be interpreted as sensitivity or specificity because no molecular reference standard was available. eCIM was positive in 18 of the 29 mCIM-positive isolates (62.1%) and was therefore not directly comparable with assays performed on all 53 CRPA isolates.

Verma et al. similarly reported high phenotypic positivity among CRPA isolates, with DDST positive in 87.3% and a combined-disc synergy test positive in 93.2% [19]. The DDST rate in their study is broadly comparable with the 90.6% observed here. Nevertheless, agreement between phenotypic tests is not necessarily expected because DDST detects EDTA-inhibitable metallo-beta-lactamase activity, Carba NP detects carbapenem hydrolysis, and mCIM detects

inactivation of a carbapenem disc. MHT is also less specific and is no longer preferred when more reliable methods are available [6-10].

The 100% Carba NP positivity but 54.7% mCIM positivity in the present study should not be interpreted as proof that Carba NP is more sensitive. Simner et al., using molecularly characterised isolates in a multicentre evaluation, reported mean sensitivity and specificity of 97.8% and 97.8% for Carba NP and 98.0% and 95.0% for mCIM in carbapenemase-producing *P. aeruginosa* [20]. The lower mCIM positivity observed here may reflect differences in inoculum, enzyme expression, resistance mechanisms, or test execution; importantly, the present study lacked a molecular reference standard. These percentages are therefore test-positivity rates rather than estimates of sensitivity, specificity, or diagnostic accuracy. Among the 29 mCIM-positive isolates, 18 (62.1%) were eCIM positive, indicating an EDTA-inhibitable metallo-beta-lactamase phenotype. This finding is consistent with the recognised importance of metallo-beta-lactamases in Indian CRPA isolates. Ellappan et al. detected *blaVIM* and *blaNDM* in 23.1% and 17.3%, respectively, among CRPA isolates from a South Indian tertiary-care hospital [21]. More recently, Menon et al. reported that 74.1% of a South Indian *P. aeruginosa* collection was MDR, 93% of the MDR isolates were meropenem resistant, and carbapenem resistance was associated with *oprD* loss-of-function mutations and a high prevalence of *blaNDM-1* [22]. These comparisons also emphasise that phenotypic EDTA inhibition cannot identify a specific gene. PCR or sequencing for *blaVIM*, *blaIMP*, *blaNDM*, and other carbapenemase genes would be required to establish the underlying mechanisms in the present isolates.

Cinnamomum verum bark extract showed strong experimental activity: 122 of 132 MDR isolates (92.4%) were classified as very active and the remaining 10 (7.6%) as active according to the study-defined zone categories. This finding is in line with the in vitro antibacterial and antibiofilm effects reported by Wijesinghe et al. for *C. verum* leaf oil and by Utcharyakiat et al. for cinnamon bark oil and cinnamaldehyde against *P. aeruginosa* [12,13]. Kalia et al. and Topa et al. also demonstrated interference with quorum-sensing-regulated virulence and biofilm-related phenotypes [14,15]. Meccatti et al. further observed that standardised *C. verum* extracts, particularly in combination with Brazilian green propolis, could inhibit biofilm formation and reduce established *P. aeruginosa* biofilms [23]. Direct numerical comparison remains difficult because essential oils, hydroethanolic extracts, purified cinnamaldehyde, and combined preparations differ in chemical composition, concentration, solvent, and test method.

The reported MIC range of 0.5-2 mg/mL and MBC range of 1-4 mg/mL support an antibacterial effect under the experimental conditions, but these values should not be translated into clinical susceptibility categories because validated breakpoints for *C. verum* do not exist. In addition, Tetard et al. showed that subinhibitory cinnamaldehyde exposure can transiently increase expression of several Mex efflux systems and reduce susceptibility to conventional antipseudomonal agents [16]. The extract should therefore be considered an investigational in vitro candidate rather than an alternative therapy. Botanical authentication, extraction yield, chemical profiling, solvent controls, reproducibility testing, cytotoxicity assessment, pharmacokinetic studies, and in vivo evaluation are required before clinical relevance can be established. Overall, the findings demonstrate a considerable proportion of carbapenem resistance within an MDR-enriched *P. aeruginosa* cohort, substantial discordance among phenotypic carbapenemase-associated assays, and promising but preliminary in vitro activity of *C. verum*. The study contributes useful local laboratory data, but molecular confirmation and standardised plant-extract testing are essential for strengthening the interpretation and comparability of these results.

Limitations

This study has several limitations. This study was conducted at a single tertiary-care centre and included only MDR *Pseudomonas aeruginosa* isolates, which may limit the generalisability of the findings. The absence of molecular testing precluded confirmation of specific carbapenemase genes and assessment of the diagnostic accuracy of the phenotypic assays. Clinical risk factors and treatment outcomes were not evaluated. Furthermore, the *Cinnamomum verum* extract was not chemically standardised; therefore, its in vitro antibacterial activity should not be extrapolated to clinical efficacy.

CONCLUSION

Carbapenem resistance was common among the MDR *Pseudomonas aeruginosa* isolates examined. The different positivity rates obtained with DDST, MHT, Carba NP, mCIM, and eCIM demonstrate that these phenotypic assays are not interchangeable and should be interpreted according to their individual principles. Molecular confirmation is necessary to identify the underlying carbapenemase genes and assess the diagnostic performance of these tests. *Cinnamomum verum* bark extract demonstrated promising in vitro antibacterial activity against both MDR and carbapenem-resistant isolates. However, these findings remain preliminary and should not be interpreted as evidence of clinical efficacy. Further studies using chemically standardized extracts, molecular characterization, cytotoxicity assessment, and in vivo and clinical evaluation are required before any therapeutic application can be considered.

Declarations

Ethics approval: The study received clearance from the Institutional Ethics Committee of Index Medical College Hospital and Research Center, affiliated with Malwanchal University, under approval number MU/Research/EC/Ph.D./2023/300.

Conflicts of interest: There is no any conflict of interest associated with this study.

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors & contributions: Author equally contributed the work.

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