Research Article

# Antimicrobial Potential of *Solanum virginianum* L against Carbapenem Resistant of *Acinetobacter baumannii* and *Klebsiella pneumoniae*

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**Received:** 11-10-2024; **Revised:** 23-12-2024; **Accepted:** 05-01-2025; **Published online:** 20-01-2025.

**ABSTRACT**

**Background:** Antimicrobial resistance, particularly from carbapenem-resistant strains such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*, poses a significant public health threat.

**Aims/Objectives**: This study investigates the phenotypic and genotypic characteristics of these pathogens and evaluates the antibacterial potential of *Solanum virginianum* extracts.

**Methods:** Clinical samples were cultured on standard media for phenotypic identification. Carbapenemase production was detected using the CARBA NP method. Genomic DNA was extracted, and PCR assays targeted resistance genes (blaKPC, blaNDM, blaVIM, blaOXA-48, blaIMP). Additionally, *S. virginianum* was identified and extracted using methanol, and its antibacterial efficacy was evaluated through agar well diffusion against the identified pathogens.

**Results:** *A. baumannii* and *K. pneumoniae* were confirmed as carbapenem-resistant, with positive PCR results for resistance genes. The methanol extract of *S. virginianum* exhibited moderate antibacterial activity, with mean inhibition zones in cm of 1.47 ± 0.12 for *A. baumannii* and 1.72 ± 0.06 for K. pneumoniae, compared to effects from standard antibiotics.

**Conclusion:** The study successfully identifies carbapenem-resistant pathogens and confirms the presence of resistance genes. These findings point to the urgent need for continued monitoring of antibiotic resistance and exploration of plant-based alternatives in treating resistant infections.

**Keywords:** Antimicrobial activity, *Solanum virginianum L*, Carbapenem resistant, *Acinetobacter baumannii* and *Klebsiella pneumoniae.*

## INTRODUCTION

Carbapenem resistant gram-negative bacterial infections represent a major clinical concern. Increasing occurrence of infections is seen recently due to carbapenem resistant organism especially in *K. pnuemoniae* and *A. baumanii*.[1-3]

*K. pneumoniae* and *Acinetobacter baumanii* are associated with hospital acquired infections and Infectious Diseases Society of America (IDSA) listed this bacterium as one of the top-priority dangerous microorganisms. *K. pneumoniae* along with A. baumanii are two important multidrug-resistant and also carbapenem-resistant pathogens in the health care.[4] Resistance to carbapenem is mediated by plasmid-coded carbapenemase, which has emerged globally and has become a major worry. The activity detection of carbapenemases has a very strong impact on the control of hospital infections, as their presence can initiate measures to prevent lateral spread of resistance and potential outbreaks.[5,6]

For the treatment of multidrug resistance (MDR) microbes colistin and tigecycline are the only active antibiotics. While tigecycline has been recommended for problematical intra-abdominal infections, skin infections, and community-acquired pneumonia. However latest meta-analysis has shown that tigecycline is no better than the widely used antibiotics. More disappointingly in many countries tigecycline is commercially available. As a result, treating physicians started using an 'old' drug, colistin that was used clinically in the late 1950s.[7-9] The use of colistin has been systemically limited, primarily due to reports of severe nephrotoxicity of colistin and the production of alternative, less toxic antibiotics. Researchers have re-evaluated the toxicity of colistin and have found that the occurrence of toxicity arising from the use of colistin is less severe and significant were reported.[10-12]

Solanum, a genus of plants, includes herbs, shrubs, and trees. It grows globally, particularly in sandy soils and is known as yellow-berried nightshade. Leaves can be entire, lobed, or pinnatifid, and flowers are found in cymes. The fruit is either a globose or elongated berry.[13-15] The plant has thorny calyx and violet corolla. The plant is used in Ayurveda, treating chest pain, asthma, scorpion bite, leucoderma, and sterility. Its roots, oil, and ash have antimicrobial, antioxidant, and anti-inflammatory properties, and are also used for toothache relief.[16-20] In this study, to identify the potential therapeutic applications of *S. virginianum* based on its antimicrobial properties.

## MATERIALS AND METHODS

The present study was conducted at the department of Microbiology, at Vedantaa Institute of Medical Sciences (VIMS) and Research Center, Dahanu, Maharashtra, India. The research exclusively involved the use of microbial isolates obtained from clinical samples. No human subjects or animal experimentation were involved in this study. All samples were collected and processed in accordance with institutional protocols, ensuring adherence to relevant biosafety and ethical guidelines.

### Phenotypic identification

The clinical samples such as Pus, BAL, Blood and Body fluids received were processed on standard culture media (i.e. streaked on, Blood agar and MacConkey agar), Colonies were later subjected to biochemical tests such as catalase, oxidase, indole production, urease, utilization of citrate, TSI test as per standard guidelines. Which enables for the phenotypic identification of the organism.

### Detection of carbapenemases by CARBA NP method

Carbapenem resistant isolates of *Acinetobacter baumannii* and Klebsiella pneumonia were collected and subjected to Carba NP, CarbAcineto NP test and Carbapenem inactivation method (mCIM) respectively.

### DNA extraction

Colonies of carbapenemases resistance *Acinetobacter baumannii* and Klebsiella pneumonia were cultured in nutrient broth for 24 hours at 37 °C, with occasional shaking and a 5-minute microcentrifuge spin at the maximum speed of 1000 rpm. After that, the cell suspension was pipetted and centrifuged at 4000 rpm for 10 min in a separate test tube. Total genomic DNA was isolated from using boiling lysis method by re-suspending at cell pellet of 1 g in 50UL water which were then boiled for 15 minutes at a temperature of 102 to110 degrees Celsius. The cooling was performed on ice to the sample place and for 1 min to 5000 rpm it was centrifuged. The supernatant was re-suspended in cold 95% ethanol and kept at -20 °C until PCR was performed.

### Polymerase chain reaction (PCR) assay

PCR mix contained 50 ul of distilled water, Buffer 1x, MgCl2, Taq polymerase fields, dNTP pump, primers encoding target gene such as $bla_{KPC}$, $bla_{NDM}$, $bla_{VIM}$, $bla_{OXA-48}$ and $bla_{IMP}$ (Table 1).

**Table 1**: Primers for beta-lactamase genes associated with antibiotic resistance

| Target Gene | Forward Primer (F) | Reverse Primer (R) | Associated Organism |
|---|---|---|---|
| blaKPC | 5'-AAGCTTATTACGAGTCTGCTG-3' | 5'-CCTCAGGTCAGTGTCATTTG-3' | *Klebsiella pneumoniae* |
| blaNDM | 5'-GGTTTGGCGCAACTTCA-3' | 5'-GCGGCCGTTTTCAGTG-3' | *Klebsiella pneumoniae, Acinetobacter baumannii* |
| blaVIM | 5'-GCCAGCAGTACCAAGGAC-3' | 5'-CCGAGTGAAGCCTGACAT-3' | *Acinetobacter baumannii* |
| blaOXA-48 | 5'-ATGGAAGTTTATCGTGCCTT-3' | 5'-TTACTGATCGTTTTCGCCAC-3' | *Acinetobacter baumannii* |
| blaIMP | 5'-GCTGCCCGCCTGCC-3' | 5'-CGGATCCACGACGTC-3' | *Acinetobacter baumannii* |

Targets are identified using four different dyes (FAM/Green, HEX/Yellow, Texas Red/Orange, and Cy5/Red) and was prepared in a 0.2-ml tube. A PCR mixture was prepared in 20ul volume and 10ul DNA template was added to the PCR mixture and placed inside the Insta Q96 (Himedia) thermal cycler for the amplification process This process comprised initial denaturation at $92^0$C for 30 minutes, denaturation at $92^0$C for 10 minutes, annealing at $56^0$C for 10 min and extension at $72^0$C for another 10 min.

### Collection and identification of plant

The plant was collected at Vedantaa Institute of Medical Sciences and Research Center, Dahanu, campus during September 2024. The plant was identified based on its characteristics using the web tool https://identify.plantnet.org/.

### Plant Extraction

This study included whole plants (roots, stems, leaves, flowers, and fruits). To make the plant broth solution. 15 g of cleaned chopped plant materials were blended in a 250-ml flask with 20 ml of methanol for 48 hrs with occasional stirrings. The crude extract was stored in the refrigerator until use. The yield and color of extract were dark green, respectively.

### Antibacterial activity of methanol extract of S. virginianum

The extract's antibacterial potential was assessed against two Gram negative bacteria namely, *Acinetobacter baumannii* and *Klebsiella pneumoniae* were isolated from VIMS tertiary care hospital. The test bacteria were maintained in pure cultures on nutrient agar slants and kept in a refrigerator. Broth cultures of the test bacteria were performed by aseptically transferring a pure culture of the test bacteria to nutrient broth tubes and then incubating the tubes overnight at 37°C. The sensitivity of the broth cultures to plant extract was evaluated by agar well diffusion. On sterile nutrient agar plates, the broth cultures were aseptically inoculated using swabs. Using a cork borer 6 mm diameter, 4 different wells were formed and filled with 100 µl of extract, chloramphenicol (reference antibiotic mg/ml), 100 µl sterile distilled water, and Dimethyl Sulfoxide (DMSO). The plates were allowed to stand for 30 minutes and then incubated at 37°C for 48 hours. After incubation, the zones of inhibition around the wells were measured.

## RESULTS AND DISCUSSION

### Phenotypic identification of Carbapenem Resistant

*Acinetobacter baumannii* on MacConkey agar showing a pink to light lavender appearance. Acinetobacter produced a unique brown discoloration of heart infusion agar with blood agar into which glucose was incorporated. *A. baumannii* producing a brown diffusion pigment on MacConkey agar. It is oxidase-negative and non-motile, both typical characteristics of this species. The organism was grow at 42°C, demonstrating its resilience to higher temperatures. Additionally, it ferments glucose, which aligns with the biochemical profile of *A. baumannii*. However, it does not reduce nitrate. The variable results for gelatin hydrolysis and urease production, along with the lack of pigmentation, further support the identification of this significant pathogen.[9,10]

The test results was suggest the organism was *Klebsiella pneumoniae*. The negative indole and methyl red tests are consistent with this identification, as *K. pneumoniae* typically does not produce indole and has a neutral fermentation profile. The positive Voges-Proskauer test indicates that the organism can produce acetoin, a common trait in *K. pneumoniae*. The organism also shows positive results for urease and lysine decarboxylation, further supporting its identification, as these traits are characteristic of *K. pneumoniae*. Additionally, the positive ONPG and malonate tests indicate the organism's ability to utilize these substrates, and the ability to grow at 41°C demonstrates its adaptability to higher temperatures.[11,12]

### Confirmed Carbapenem Resistant gene by RT-PCR

The RT-PCR analysis confirmed the presence of carbapenem-resistant genes in the samples tested. The target genes identified include blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP, which are associated with resistance in organisms such as *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Specific primers were used for amplification, leading to successful detection of these genes, indicating carbapenem resistance. This finding highlights the importance of monitoring these resistance mechanisms in clinical settings.[12]

**Figure 1**: *Solanum virginianum* L. plant

### Identification of plant

The plant was identified based on its characteristics using the web tool https://identify.plantnet.org/. Regarding Solanum virginianum L. (Figure 1), the analysis revealed that the identified sequences demonstrate 95.2% similarity to known sequences of this plant species. This high level of similarity indicates a close genetic relationship, suggesting that the samples was likely from *Solanum virginianum* or a closely related species. Such findings was significant for understanding the taxonomy and evolutionary relationships within the *Solanum* genus.[13,14]

### Antibacterial activity of an extract of S. virginianum

For *Acinetobacter baumannii*, the extract shows an inhibitory effect with a mean value of 1.47 ± 0.12, suggesting moderate effectiveness. In contrast, the antibiotics demonstrate a much stronger inhibitory effect with a value of 3.57 ± 0.17. This disparity indicates that while the extract can provide some level of inhibition, it is significantly less potent than the antibiotics tested. The DMSO, used as a solvent, shows no inhibitory effect (0.00 ± 0.00), which aligns with expectations, as DMSO is not typically expected to impact bacterial growth.

In the case of *Klebsiella pneumoniae*, the extract yields a slightly higher inhibitory value of 1.72 ± 0.06 compared to *A. baumannii*, indicating that this strain may be marginally more susceptible to the extract. However, the antibiotics still exhibit a strong effect, with a value of 2.86 ± 0.12, which is less than that observed for *A. baumannii*. This suggests that *K. pneumoniae* may be somewhat more resilient to the antibiotics used, although they are still effective. Again, DMSO shows no impact on bacterial growth (Table 2).[14,15]

**Table 2**: Antibacterial activity of methanol extract of *Solanum virginianum*

| | Zone of inhibition in cm | | |
|---|---|---|---|
| **Test bacteria** | **Extract** | **Antibiotics** | **DMSO** |
| *Acinetobacter baumannii* | 1.47 ± 0.12 | 3.57 ± 0.17 | 0.00 ± 0.00 |
| *Klebsiella pneumoniae* | 1.72 ± 0.06 | 2.86 ± 0.12 | 0.00 ± 0.00 |

## CONCLUSION

This study successfully identified carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* from clinical samples, confirming their resistance through the detection of key carbapenemase genes via RT-PCR. The phenotypic characterization highlighted the unique features of these pathogens, emphasizing their adaptability and resilience in clinical environments. Furthermore, the antibacterial activity of the methanol extract of *Solanum virginianum* was evaluated, revealing moderate inhibitory effects against both bacterial strains. Although the extract was significantly effective to carbapenem resistance pathogen. These findings point to the urgent need for continued monitoring of antibiotic resistance and

exploration of plant-based alternatives in treating resistant infections. Future research should focus on isolating and characterizing the bioactive compounds within *S. virginianum* to better understand their mechanisms of action and potential therapeutic applications. This work contributes to the broader understanding of antibiotic resistance and the search for novel antimicrobial agents.

## ACKNOWLEDGEMENT

The author expresses their thanks to the Department of Microbiology, Vedantaa Institute of Medical Sciences and Research Center, Palghar, Maharashtra for extending the infrastructure and study resources.

**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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