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Antibacterial Potential of Honeybee (*Apis mellifera*) Venom against *Pseudomonas aeruginosa*

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ABSTRACT

The study investigated the antibacterial properties of honeybee venom against *P. aeruginosa*. Honeybee venom contains antibacterial compounds that can help to treat lung and urinary tract infections caused by the Gram-negative bacterium *P. aeruginosa*. A study involving 264 clinical samples from urine, sputum, and pus found that venom had potent antibacterial properties against *P. aeruginosa*. The study found that venom preparation techniques could affect antibiotic efficacy and warranted further research. The most common source of samples was pus, and wound infections, abscesses, and post-operative infections were common diagnoses. When PCR was done, a particular primer was found. A distinct band on the gel at 396 and 1600 base pairs (bp) indicates the expected size of the amplicon for the Exo A and 16S rRNA genes. The study suggests that venom can be used in combination with other drugs to combat *P. aeruginosa* and could be beneficial for treating infections caused by bacterial activity.

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INTRODUCTION

The demand for natural medicines as alternatives to synthetic antibiotics is increasing due to financial constraints and a desire for improved health outcomes (Veetilvalappil et al., 2022). However, overuse, misuse, and incomplete drug regimens have led to bacterial resistance (Rabea et al., 2025; Ijaz et al., 2019). *Pseudomonas aeruginosa*, a Gram-negative bacterium, is a significant public health concern due to its rapid development of multidrug resistance (Fernández et al., 2023).

Honeybee venom, a complex mixture of bioactive peptides and enzymes, has broad-spectrum antibacterial properties. Antibiotic resistance is a growing global health concern, causing increased morbidity and mortality rates (Awad et al., 2025). Natural products like bee venom are being investigated as alternative therapies to combat resistant bacterial strains (Uddin et al., 2016). This study will use conventional microbiological methods and PCR for precise identification of *P. aeruginosa*, enhancing specificity and reliability in confirming isolate presence (Mesaros et al. 2007). Bee venom's antimicrobial, anti-inflammatory, and immunomodulatory properties may increase host immune responses and reduce infection severity (Aurongzeb et al., 2021). Venom-derived medications are recognized for their superior bioactivity, specificity, and stability compared to synthetic alternatives (Reynolds et al., 2021). Honeybee venom (BV) contains biologically active compounds like peptides and enzymes, which have antibacterial, anti-inflammatory, and cytotoxic activities (Iorizzo et al., 2022). Melittin, the major component of BV, is particularly potent against Gram-positive and some Gram-negative bacteria, disrupting cell membranes and enhancing antibiotic effects. BV has historically been used as a supplemental medication for various ailments (Zolfagharian et al., 2016). Toxins in

venoms can be beneficial as therapeutic agents, drug design templates, and pharmacological instruments in drug development (Leandro et al., 2025). This study examined the antibacterial properties of BV and gentamicin against specific bacteria strains (Muller et al., 2007; Alsayeqh, 2025). *P. aeruginosa*, a major cause of nosocomial infections, is often linked to medical devices and hospital environments, with resistance mechanisms like biofilm formation complicating treatment strategies (El-Seedi et al., 2020).

MATERIALS AND METHODS

Collection and Preparation of Sample

Samples were collected from Victoria Hospital Bahawalpur, South Punjab, using sterile syringes or swabs (Ahsan et al., 2024). Hand extraction was used to extract HB venom from healthy *A. mellifera* populations. The venom was dried, cut, and stored at -20°C. The venom was then weighed, documented, and packed in jars. Two types of bee venom were evaluated for their antimicrobial effects on *Pseudomonas aeruginosa*, including hand-extracted and lyophilized forms, using controlled stimulation techniques.

Isolation of *Pseudomonas aeruginosa*

The study involved preparing MacConkey agar plates and Cetrimide agar plates (selective media) and incubating specimens for one to two days to isolate *P. aeruginosa* strains. Morphological and physiological tests were used to identify them. 264 *Pseudomonas* isolates were collected from clinical samples (Abbas et al., 2022).

Bacterial Straining and Culturing on different Agars

MacConkey agar medium from Oxoid company was used for identifying and isolating *P. aeruginosa,* sterilized and autoclaved. Colonies were separated and incubated at 35°C for 24 to 48 hours. Samples were streaked on Cetrimide nalidixic acid agar using sterile cotton swabs for eight hours, then incubated at 370 degrees Celsius for 24 hours.

Transfer a small amount of suspended culture to a microscope slide, cover with a thin layer, and gently flame-dry or air-dry to avoid ring formations and overheating. A colony from MacConkey and Cetrimide Agar is fixed with crystal violet, then washed with tap water. Gram's iodine is used as a mordant, then cleaned with water. The slide is then cleaned with 95% alcohol, ethanol-acetone decolorizers, and safranin. The slide is then air-dried, and the counterstain is removed. The process involves rinsing, washing, and air-drying the slide to ensure the integrity of the culture (Rasool et al., 2016).

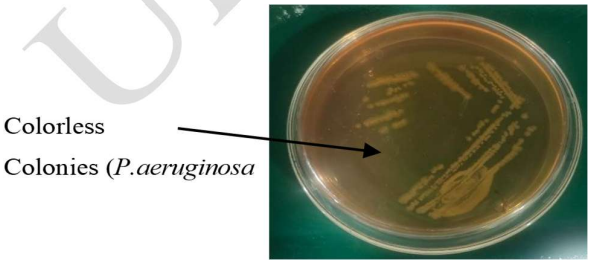

Fig 1: Colorless colonies on MacConkey agar

Subculture on Cetrimide Agar

P. aeruginosa colonies were formed on Cetrimide agar, producing two colors: pyoverdine and pyocyanin, and all 28 positive cultures were found on Cetrimide agar (Nader et al., 2021).

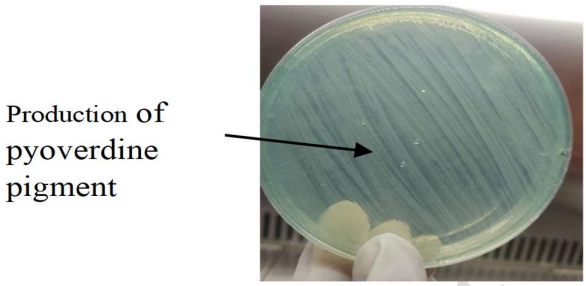

Fig 2: Results showing production of pyoverdine pigment on cetrimide agar.

Biochemical Testing

The study conducted biochemical assays on bacterial isolates, revealing that all tested positive for catalase, a enzyme that converts hydrogen peroxide into H2O and O2, and oxidase, a dark purple color indicating enzyme formation within five to ten seconds (Al-Bayati et al., 2021).

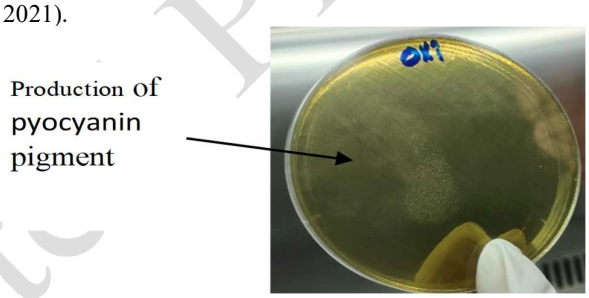

Fig 3: Results showing production of pyocyanin pigment on cetrimide agar.

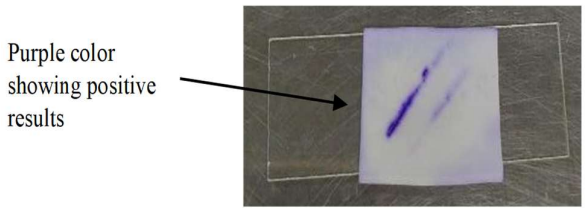

Fig 4: Colony smeared on filter paper by using Oxidase enzyme, purple hue emerged.

Oxidase test: This assay confirmed the presence of the cytochrome c oxidase enzyme, crucial in ETC, using a sterile wooden stick, filter paper, bacterial culture, petri plates, and 1% tetramethyl p-phenylenediamine dihydrochloride. Safety precautions were followed, including using fresh cultures and avoiding metal loops (Wellinghausen et al., 2025).

Catalase test: This test requires sterile swabs, glass slides, hydrogen peroxide, and bacterial culture. Fresh cultures are inoculated, and safety precautions are taken (Su et al., 2018).

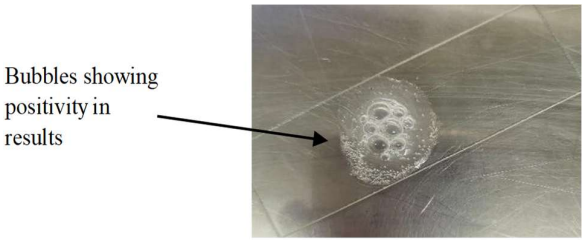

Fig 5: Bubbles showing positivity in results of on glass slide.

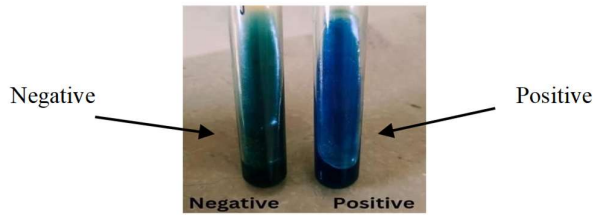

Fig 6: Green color shows negative results and blue color shows positive results.

Simmons citrate test: It involved dissolved citrate agar in distilled water, poured into angled tubes, and inserted into a bacterial needle. The test showed a blue hue, indicating alkalinity, after a full day of tilting (Su et al., 2018).

PCR

The PCR technique uses specific primers for target genes to identify amplified DNA. It involves denaturation, annealing, extension, and amplification at 95°C and 72°C (Liu et al., 2023). Results are detected by electrophoresis in an agarose gel and examined with an ultraviolet transil luminator. The size of the amplicon is determined using a 5.5% agarose gel (Garibyan et al., 2013; Basit et al., 2021).

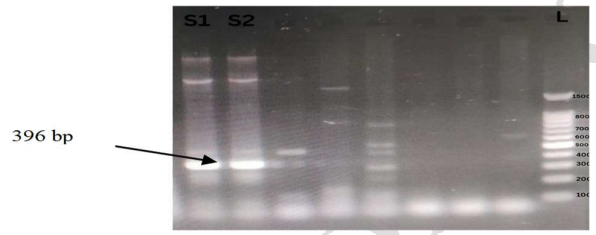

Fig 7: Agarose gel electrophoresis of a PCR amplification targeting the Exo A gene in *Pseudomonas aeruginosa.*

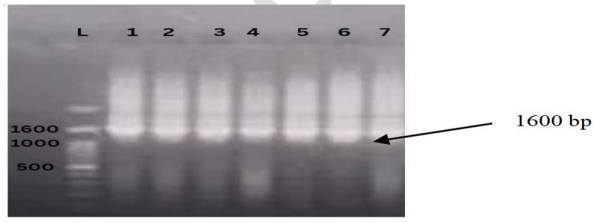

Fig 8: Agarose gel electrophoresis of a PCR amplification targeting the 16S rRNA gene in *Pseudomonas aeruginosa.*

Antibacterial Assay

Disk diffusion method used to study honey bee venom. Filter paper disks moistened in the test material are used in this manner. The venom of honey bee is put on a bacterially contaminated agar plate. The zone of inhibition exhibits increased antibacterial activity after incubation (Abdulhaq et al., 2020; Shahid et al., 2023).

MIC

The MIC of bee venom was determined using the broth microdilution method, using 96-well microplates. The bacterial inoculum concentration in each well was 5x106 CFU/ml. Ten dilutions were made, with a 1000 µg/mL stock solution made by mixing bee venom with nutrient broth. Three wells were used as background and growth controls. The MIC value was calculated using a spectrophotometer. Bacterial strains were cultivated in broth media, and the MIC of venom inhibited over 90% of the strains (El Sherry et al., 2025).

RESULTS AND DISCUSSION

Antibacterial activities of Bee venom revealed the significances opposed to *P. aeruginosa* (Gellatly et al., 2013 et al., 2013). There were different zones observed, like at 75 µg/mL 18 mm (the largest zone of inhibition), at 50 µg/mL 16 mm and at 25 µg/mL 14 mm (smaller zone) were observed in lyophilized venom. In Hand extracted venom, at 75 µg/mL 16mm (the largest zone), at 50 µg/mL 15mm and at 25 µg/mL 12mm (smaller zone) were observed (Moradali et al., 2007).

The study found *P. aeruginosa* in 10.6% of clinical samples, with the highest positivity rates in post-surgical infections, urinary tract infections, and pyelonephritis (Hanock et al., 2013). No *P. aeruginosa* isolates were found in pneumonia cases. PCR amplification confirmed culture-based identifications of *P. aeruginosa*, confirming its role in virulence and conserved sequence among clinical isolates, making it a reliable genetic marker (Lavenir et al., 2007).

The study found differential antimicrobial activity between lyophilized and hand-extracted venom, suggesting lyophilization may preserve bioactive compounds (Poirel et al., 2020). The higher prevalence of *P. aeruginosa* in hospital settings highlights the need for enhanced infection control measures (Poirel et al., 2010).

Authors' Contribution

M Jalees supervised and designed the study and M. Mahmood collected the bees for hand extraction method. Abdullah Saghir Ahmad, Muhammad Waseem Abbas, Waqas Ashraf, Zeeshan Nawaz, Qudratullah, Shah Nawaz and Roshan Riaz actively participated in preparation of manuscript, editing and finalization. Each author helped write the manuscript, checked the finished version, and gave their approval for submission.

DECLARATIONS OF COMPETING INTEREST

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Data Availability: All the data is included in this paper and may be obtained from corresponding author.

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