Microbiological and molecular characterization of a multidrug-resistant *Providencia stuartii* isolated from a diabetic wound infection

Lakshmi Sharvani K.S[1]., V.L. Asha Latha[2]., Vijaya Lakshmi D[3]., Vijaya Raghava Prasad D[4].

[1]Research Scholar [2]Assistant Professor [3]Professor [4]Professor

[1,3,4] Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

[2] Department of Biochemistry, Govt. Medical College, Kadapa, Andhra Pradesh, India

[1] Sharvanikaipa02@gmail.com, [2] vlashalatha@gmail.com [3] dvlyvu@gmail.com [4] durbaka@gmail.com

Abstract

Providencia stuartii is an opportunistic Gram-negative pathogen increasingly associated with multidrug resistance and healthcare-associated infections. In this study, we report the microbiological and molecular characterization of a multidrug-resistant *P. stuartii* isolated from a wound infection. The isolate was identified using conventional microbiological methods and confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing demonstrated resistance to multiple classes of antibiotics, including aminoglycosides, fluoroquinolones, cephalosporins, and carbapenems, with susceptibility observed only to cefepime. Phylogenetic analysis revealed close clustering with previously reported multidrug-resistant clinical isolates, underscoring the importance of accurate identification and continuous antimicrobial resistance surveillance.

Keywords: *Providencia stuartii*, multidrug resistance, wound infection, 16S rRNA sequencing, phylogenetic analysis.

I. Introduction

Providencia stuartii is a Gram-negative, facultatively anaerobic member of the family *Enterobacteriaceae* and an important opportunistic pathogen in healthcare settings[1]. The organism is intrinsically resistant to several antimicrobial agents and readily acquires additional resistance determinants through mobile genetic elements, contributing to the increasing prevalence of multidrug-resistant (MDR) *Providencia* spp. worldwide [2,4].

While *P. stuartii* is most frequently associated with urinary tract and bloodstream infections, isolation from wound environments has been increasingly reported, particularly in patients with diabetes mellitus [4,10]. Chronic wounds provide conditions that facilitate bacterial persistence, biofilm formation, and antimicrobial selective pressure, thereby promoting MDR phenotypes.

Despite its clinical relevance, microbiological and molecular data on wound-associated *P. stuartii* isolates remain scarce, particularly from the Indian subcontinent. This study aimed to characterize an MDR *P. stuartii* isolate recovered from a diabetic foot wound using phenotypic identification, antimicrobial susceptibility testing, 16S rRNA gene sequencing, and phylogenetic analysis.

II. Methods

Clinical Specimen Collection

A wound-derived bacterial isolate was recovered from a diabetic patient presenting with a non-healing foot wound. Deep wound swabs were collected following debridement and transported to the microbiology laboratory under sterile conditions [5].

Microbiological Culture and Identification

Specimens were inoculated onto blood agar and MacConkey agar and incubated aerobically at 35°C for 24 hours. Colony morphology was recorded. Preliminary identification was performed using conventional biochemical tests including urease, citrate utilization, indole production, and motility [5,9].

Species confirmation was performed by 16S rRNA gene sequencing. PCR amplification was conducted using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Sanger sequencing results were compared with GenBank using BLAST. The sequence was submitted to NCBI (Accession number: OR636879).

Antimicrobial Susceptibility Testing (AST)

AST was performed using the Kirby-Bauer disk diffusion method according to CLSI 2022 guidelines [7]. Tested antibiotics included amikacin, amoxicillin, cefixime, ceftazidime, tobramycin, cefotaxime, imipenem, cefepime, gentamicin, ceftriaxone, kanamycin, cefuroxime, ofloxacin, and ciprofloxacin. Zones of inhibition were interpreted as susceptible (S), intermediate (I), or resistant (R) per CLSI breakpoints [7]. Colistin susceptibility testing was not performed due to the limitations of disk diffusion methods.

Phylogenetic Analysis

The 16S rRNA sequence was aligned with reference sequences from GenBank. A neighbor-joining phylogenetic tree with 1,000 bootstrap replications was constructed using MEGA 11 software, to determine the evolutionary relationships of the isolate with other MDR *Providencia stuartii* strains [4].

III. Results

Microbiological Characterization

On MacConkey agar, the isolate produced pale-pink, non-lactose-fermenting colonies, as shown in Figure 1A, consistent with characteristics of non-lactose-fermenting members of the Enterobacteriaceae family. Gram staining demonstrated Gram-negative bacilli (Figure 1B). Further biochemical characterization revealed a positive urease reaction and citrate utilization, while the isolate tested negative for indole production. These phenotypic and biochemical features were consistent with the preliminary identification of *Providencia stuartii*, which was subsequently confirmed by molecular analysis.

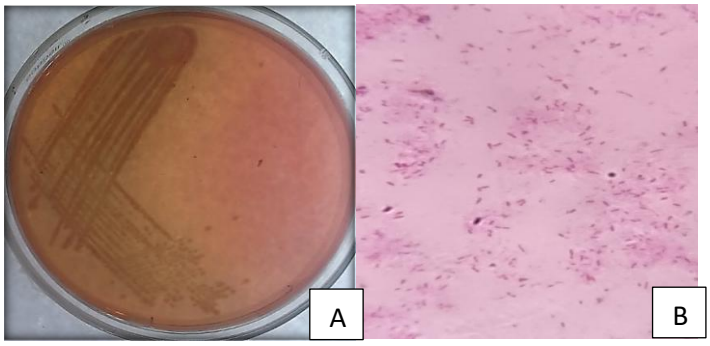

Figure 1: A) *Providencia stuartii* culture on MacConkey agar, B) Gram staining showing Gram-negative bacilli

Antimicrobial Susceptibility

Antimicrobial susceptibility testing demonstrated that the isolate exhibited a multidrug-resistant (MDR) phenotype, with susceptibility observed only to cefepime. The isolate showed resistance to multiple classes of antimicrobial agents, including aminoglycosides, fluoroquinolones, cephalosporins, and carbapenems. This extensive resistance profile underscores the limited therapeutic options available for infections caused by *Providencia stuartii*. The detailed antimicrobial resistance pattern of the isolate is summarized in Table 1.

S.No	Antibiotic	Wound culture
1	Amikacin	R
2	Amoxycillin	R
3	Cefixime	R
4	Ceftazidime	I
5	Tobramycin	R
6	Cefotaxime	R
7	Imipenem	R
8	Cefepime	S
9	Gentamicin	R
10	Ceftriaxone	R
11	Kanamycin	R
12	Cefuroxime	R
13	Ofloxacin	R
14	Ciprofloxacin	R

Table 1: Antimicrobial susceptibility pattern of *Providencia stuartii*, R- Resistant, S- Susceptible, I- Intermediate.

Molecular Identification and Phylogeny

16S rRNA gene sequencing confirmed the identity of the isolate as *Providencia stuartii*, showing a 99.28% sequence similarity with reference sequences available in the GenBank database. Phylogenetic analysis revealed that the isolate clustered closely with previously reported multidrug-resistant (MDR) clinical strains of *P. stuartii*, indicating a close evolutionary relationship. This clustering pattern is consistent with earlier reports of MDR *P. stuartii* isolates and highlights the importance of molecular tools in accurate species identification and epidemiological surveillance(Figure 2).

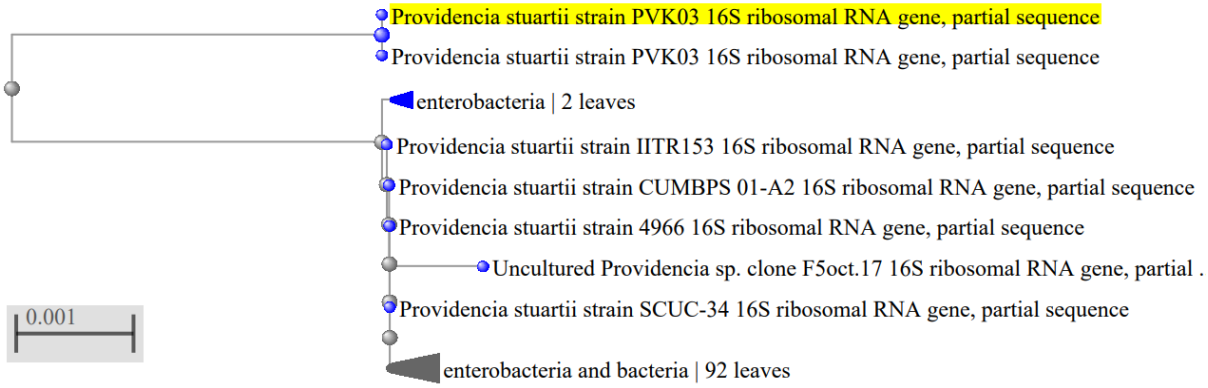

Figure 2: Phylogenetic tree of *Providencia stuartii* with closely related genomes.

IV. Discussion

Providencia stuartii is an opportunistic Gram-negative bacillus increasingly recognized in both nosocomial and community infections [2,3]. While urinary tract and bloodstream infections are most frequently reported, wound infections, particularly in patients with diabetes mellitus, remain uncommon and pose therapeutic challenges due to multidrug-resistant (MDR) profiles [4,10].

The isolate in this study exhibited resistance to aminoglycosides, fluoroquinolones, third-generation cephalosporins, and carbapenems, with retained susceptibility to cefepime. This resistance pattern aligns with global trends of extensively drug-resistant P. stuartii and highlights the challenges in selecting effective empiric therapy [8,9]. MDR mechanisms in P. stuartii include intrinsic resistance, extended-spectrum β-lactamases, plasmid-mediated carbapenemases, and other mobile genetic elements [5,6,7].

Molecular identification via 16S rRNA gene sequencing confirmed species-level identification and mitigated the risk of misidentification inherent in phenotypic methods [1,11]. Phylogenetic analysis revealed that the isolate clusters with previously reported MDR clinical P. stuartii strains from India and other regions, with bootstrap support exceeding 90%. The clustering pattern may correlate with the observed multidrug-resistant phenotype, suggesting conserved lineage features among hospital-associated strains[7,11]. The isolate was distinct from environmental strains included in the analysis, further supporting its clinical origin.

Although virulence factors and resistance genes were not characterized in this study, previous reports indicate that wound-derived P. stuartii strains possess biofilm-associated and resistance determinants that enhance persistence in chronic wound environments [4,7]. A limitation of the present study is the absence of whole-genome sequencing or targeted resistance gene profiling, which would have provided a more detailed understanding of resistance mechanisms.

Nevertheless, this brief communication contributes to limited data on wound-associated MDR P. stuartii in India. Accurate laboratory identification, antimicrobial susceptibility testing, and molecular analysis are essential for surveillance, infection control, and guiding therapy in healthcare settings.

V. Conclusion:

This brief communication describes the microbiological and molecular characterization of a multidrug-resistant *Providencia stuartii* isolate recovered from a wound infection. Accurate laboratory identification and antimicrobial susceptibility testing remain essential for surveillance and infection control, particularly in the context of emerging resistance among opportunistic pathogens.

VI. Declarations:

Funding:

This study was a part of the project supported by Rashtriya Uchchattar Shiksha Abhiyan (RUSA) (File No: RUSA-ANU/YVU/Research project-24/Sanction order/2024)

Ethical approval:

The work has been conducted at the Department of Microbiology, Yogi Vemana University, Kadapa, after receiving ethical approval from the Institutional Ethical Committee (IEC) (Ref. No. 01/IEC/DVR/YVU/2022-23, dated 24.04.2023).

Author Contributions:

Author Contribution Statement; Lakshmi Sharvani K.S has carried out the experiments and prepared the original draft. Vijaya Lakshmi D and Vijaya Raghava Prasad D conceived, planned and supervised the study. VL Asha Latha helped in the collection of the samples. All authors have read and approved the final manuscript.

Acknowledgements:

The authors would like to acknowledge Rashtriya Uchchattar Shiksha Abhiyan (RUSA) for funding, additionally, the Department of Microbiology at Yogi Vemana University in Kadapa for their assistance during the research

Consent for Publication:

Written informed consent was obtained from the patient for publication and the subject of this case report is not identifiable in photographs.

Availability of Data and Materials:

The authors confirm that all data and information supporting the findings of this study are available within the article.

Competing Interests:

The authors declare no competing interests

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