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RESEARCH ARTICLE

First report of multidrug-resistant *Pseudomonas juntendi* from an equine suppurative lesion

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ABSTRACT

Pseudomonas juntendi is a recently identified opportunistic pathogen belonging to the *Pseudomonas putida* group. Strains of *P. juntendi* are frequently associated with multidrug resistance, posing significant challenges in the clinical management of associated disease condition. To the best of our knowledge, this is the first report describing the isolation and identification of *P. juntendi* from a horse. In the present study, a horse presented with swelling in the neck region accompanied by purulent discharge was investigated. A pus sample was aseptically collected and subjected to bacteriological isolation and biochemical characterization. Bacteriological examination revealed the presence of an aerobic, Gram-negative, non-spore-forming, non-motile, rod-shaped bacterium. Biochemically, the isolate tested positive for catalase, cytochrome oxidase, and citrate utilization, while negative reactions were observed for urease, methyl red, Voges–Proskauer, indole production, motility, and triple sugar iron tests. Based on the phenotypic and biochemical characteristics, the organism was preliminarily identified as a Pseudomonas species. For molecular confirmation, 16S rRNA gene sequencing was performed. Sequence analysis confirmed that the isolate belonged to the *Pseudomonas putida* group and was identified as *Pseudomonas juntendi*. Antimicrobial susceptibility testing against 17 antibiotics was carried out using the disc diffusion method. The isolate exhibited resistance to eight antibiotics, thereby demonstrating a multidrug-resistant phenotype. In this study, we report the first isolation of *Pseudomonas juntendi* from a suppurative lesion on the neck of a horse.

Keywords: *Pseudomonas juntendi*, Horse, Pus, 16S rRNA sequencing

INTRODUCTION

Pseudomonas juntendi is an opportunistic bacterial pathogen notable for its resilience across diverse environments, versatile metabolic capabilities, rapid growth, and high tolerance to oxidative stress and toxic compounds[1]. Recently, *P. juntendi* has been recognized as a novel member of the *Pseudomonas putida* group, which includes species such as *P. alloputida, P. anuradhapurensis, P. asiatica, P. fulva, P. inefficax, P. kurunegalensis, P. monteilii, P. oryzicola, P. parafulva, P. putida, P. pyomelaninifaciens, P. persica*, and *P. shirazica*[2].

P. juntendi is an aerobic, Gram-negative, non-spore-forming, motile, rod-shaped bacterium measuring approximately 1.0–2.0 μm in length and 0.5–0.8 μm in width[3]. It can grow within a temperature range of 4–40 °C and tolerate NaCl concentrations of 1.0–7.0%. The organism was initially identified as a potential human pathogen[4]. Although it has been isolated from various clinical specimens in Japan and Myanmar[5], its role as an animal pathogen remains unclear and warrants further investigation.

Previous studies have established *P. aeruginosa* as a significant pathogen causing urinary tract infections and exhibiting antimicrobial resistance, highlighting the pathogenic potential of other *Pseudomonas* species[6]. However, *P. juntendi* remains an understudied member of the *P. putida* group, particularly with respect to antimicrobial resistance[7]. Therefore, the present study aimed to detect the presence of *P. juntendi* in a pus sample from a horse and to evaluate its antimicrobial susceptibility profile.

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MATERIALS AND METHODS

Sample collection

Pus sample was collected aseptically from the neck swelling in a horse using a sterile swab at the Veterinary Clinical Complex, DGCN COVAS, CSKHPKV, Palampur. The swab was transported in phosphate-buffered saline (PBS) on ice to the Bacteriology Laboratory, Department of Veterinary Microbiology, and processed immediately for bacterial isolation.

Isolation and identification of *P. juntendi*

The pus sample was inoculated onto blood agar and incubated at 37 °C for 24 hours. Representative colonies were subjected to Gram staining and standard biochemical tests for identification, following the methodology described by Wulandari *et al*. (2019)[8]. Biochemical characterization included catalase, oxidase, nitrate reduction, Triple Sugar Iron (TSI), sulfide indole motility (SIM), methyl red (MR), Voges–Proskauer (VP), indole, and oxidative-fermentative (O/F) tests.

DNA extraction and 16S rRNA sequencing

Genomic DNA was extracted from purified bacterial colonies using the DNeasy blood and tissue kit (Qiagen). The extracted DNA was sent to Eurofins Genomics India Pvt. Ltd., Bangalore, for 16S rRNA gene amplification and sequencing. Universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used[9]. The obtained sequences were analyzed using BLASTn against the NCBI nucleotide database for species identification based on sequence homology.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method[10] against 17 antibiotics: amikacin (AK) (10 µg), ampicillin (AMP) (10 µg), amoxicillin (AMX) (30 µg), amoxicillin/clavulanic acid (AMC) (30 µg), cefepime (CPM) (30 µg), ceftriaxone (CTR) (30 µg), chloramphenicol (C) (30 µg), cloxacillin (COX) (5 µg), cotrimoxazole (COT) (25 µg), imipenem (IPM) (10 µg), meropenem (MRP) (10 µg), penicillin-G (P) (2 units), piperacillin/tazobactam (PIT) (100/10 µg), polymyxin B (50 units), gentamicin (GEN) (30 µg), neomycin (N) (30 µg), and streptomycin (S) 10 µg), following CLSI guidelines[11].

RESULTS

Clinical signs and symptoms

A prominent swelling (appx. 4-5 cm in diameter) on the ventral aspect of the neck and mandibular region (persisting for 15 days) of a horse was present with a fistula in the center. The yellowish purulent discharge was coming out of the fistula (Fig. 1A). The animal was lethargic, showed inappetence and exhibited severe pain at the wound site.

Isolation of *P. juntendi*

Pure cream colored bacterial cultures with non-hemolytic, circular, and convex colonies were obtained on blood agar after 24 hours of incubation at 37 °C (Fig. 1B). On brain heart infusion (BHI) agar, colonies were characteristic cream color, circular, medium-sized, with entire margins, raised elevation, and smooth consistency (Fig. 1C). Gram's staining revealed presence of gram-negative bacilli with solitary arrangement (Fig. 1D).

Biochemical characterization of *P. juntendi*

Biochemical tests showed that the *P. juntendi* isolate was positive for catalase, cytochrome oxidase, and citrate utilization, while it tested negative for urease, MR, VP, indole and TSI tests (Fig. 2). The isolate exhibited an oxidative metabolism in Hugh–Leifson's oxidative/fermentative (O/F) medium, indicating an aerobic nature.

Sequencing

The sequence of 16S rRNA gene of the *P. juntendi* was received and edited by using DNA Star Bioinformatics tool. The sequence was analyzed for their similarity with previously published sequences in NCBI by BLASTn module available on the NCBI and it was observed that the sequence showed 100% similarity with the *P. juntendi* isolates sequences reported from other countries. The 16S rRNA sequence of *P. juntendi* has been submitted in the NCBI database (Accession no. PX453077).

Antimicrobial susceptibility testing

The results suggest that the *P. juntendi* bacterial strain exhibited resistant to amoxicillin, amoxicillin/clavulanic acid, ampicillin, co-trimoxazole, chloramphenicol, cloxacillin, penicillin, polymyxin B, and meropenem. However, the isolate was found sensitive to gentamicin,

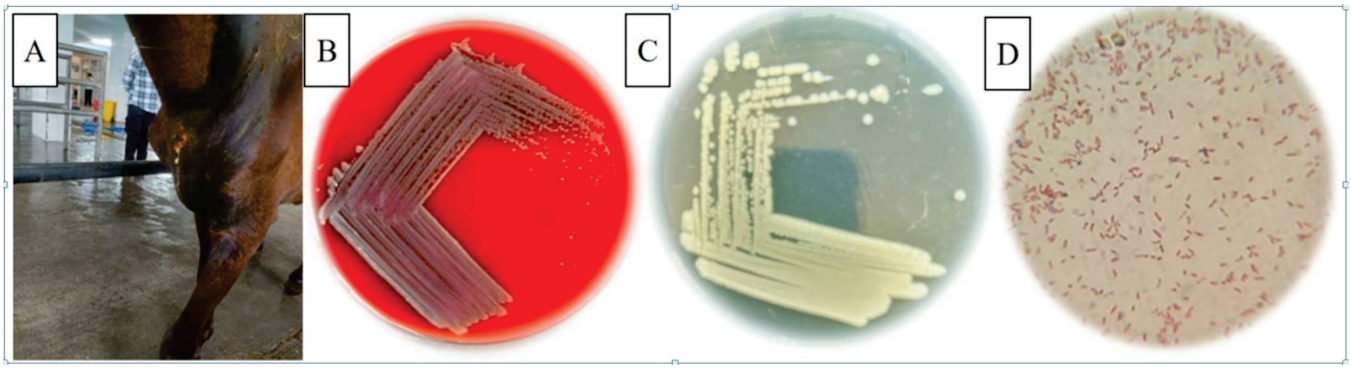

Fig. 1. (A) Purulent discharge from fistular track in the swelling at base of neck of horse; **(B)** Non-hemolytic colonies of *P. juntendi* on 5% sheep blood agar; **(C)** *P. juntendi* colony morphology on Brain Heart Infusion (BHI) agar; **(D)** Gram's stained *P. juntendi* organisms.

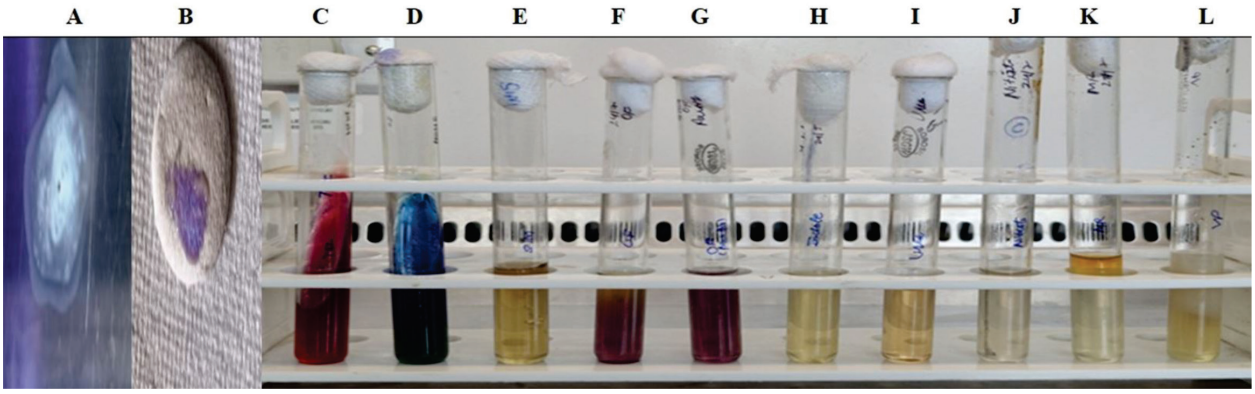

Fig.2. Biochemical characterization of *P. juntendi* **(A)** Catalase positive, **(B)** Oxidase Positive; **(C)** Triple Sugar Iron agar Negative, **(D)** Citrate Positive; **(E)** SIM: Non-motile **(E,F,G)** O/F Test: Oxidative, **(H)** Indole Negative; **(I)** Urea Negative; **(J)** Nitrate Negative; **(K)** MR Negative; **(L)** VP Negative)

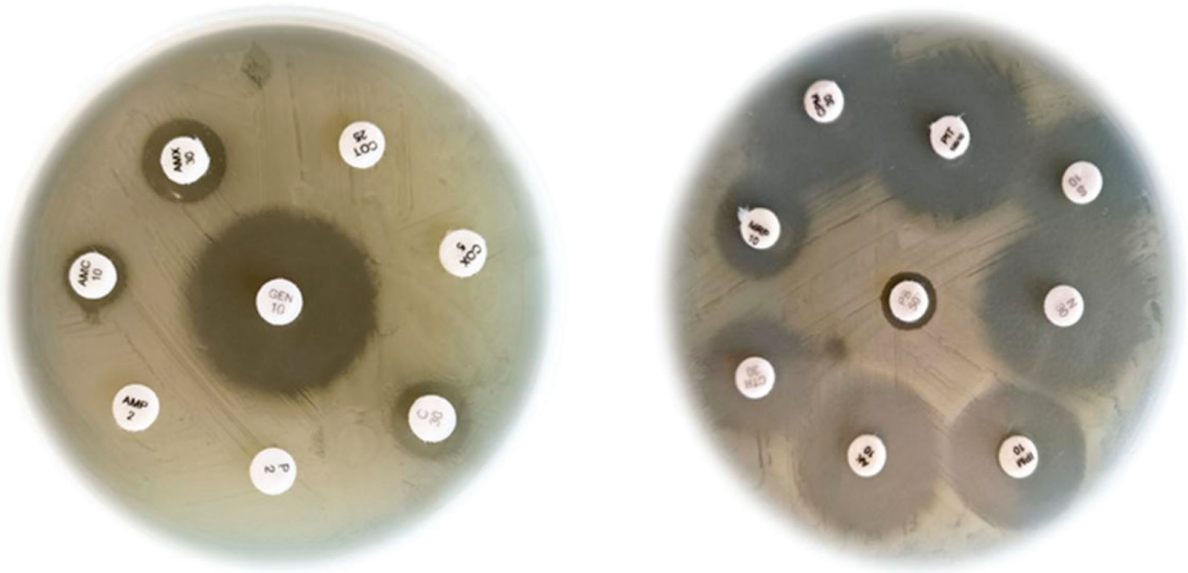

Fig. 3. Antimicrobial susceptibility testing of *P. juntendi* showing resistance to ampicillin, amoxicillin, amoxicillin/clavulanic acid, co-trimoxazole, cloxacillin, chloramphenicol, meropenem, penicillin and polymyxin B.

ceftriaxone, cefepime, streptomycin, imipenem, amikacin, piperacillin/tazobactam and neomycin.

DISCUSSION

P. juntendi is an opportunistic pathogen capable of harboring multiple antimicrobial resistance genes, contributing to persistent infections and reduced therapeutic efficacy. In the present study, pure colonies of *P. juntendi* were obtained from a suppurative lesion on the neck of a horse. The isolate showed no pigment production on BHI agar, which aligns with the previous reports indicating *P. juntendi* forms protruding colonies with smooth and regular edges, non-confluent growth and lacks pyocyanin production after overnight incubation at 37°C [5]. Gram's staining confirmed the presence of Gram-negative bacilli, consistent with in agreement with earlier findings for *Pseudomonas group*[12].

Biochemical characterization results were also consistent with previous reports[13]. Although *P. juntendi* is generally reported as motile[3], the isolate in this study was non-motile, suggesting possible phenotypic variation. Phylogenetic analysis based on 16S rRNA sequencing showed that *Pseudomonas* species comprise 13 distinct groups, including *P. putida*, *P. aeruginosa*, *P. fluorescens*, and *P. syringae*[14]. The 16S rRNA gene is widely used for bacterial identification due to the presence of conserved and variable regions[15]. BLASTn analysis revealed, 100% sequence similarity of the isolate with *P. juntendi* strain Sample 9-9 (CP125214.1) and *P. putida* strain IMV B-7289 (MZ373330.1), confirming its taxonomic affiliation.

The *P. putida* group is considered a potential reservoir for antimicrobial resistance (AMR) genes[16] and may facilitate horizontal gene transfer[17]. The isolate exhibited resistance to co-trimoxazole, cloxacillin, penicillin, amoxicillin, polymyxin B, ampicillin, amoxicillin/clavulanic acid and meropenem. It was sensitive to gentamicin, ceftriaxone, cefepime, streptomycin, imipenem, amikacin, piperacillin/tazobactam and neomycin. Based on resistance to multiple antibiotics, the isolate can be classified as multidrug-resistant[18]. The widespread antimicrobial resistance observed in *P. juntendi* may enhance its persistence and dissemination in both animal and human populations. However, this study has limitations, as the animal was not followed up for treatment outcomes. Recent reports of clinical isolates suggest that *P. juntendi* may be an emerging pathogen rather than a rare laboratory isolate.

CONCLUSION

This study identifies *P. juntendi* as a multidrug-resistant pathogen associated with pus forming abcess in horses. Further research focusing on genomic characterization and surveillance is essential to monitor its spread in animal and human populations.

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