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Genome-based Characterization of *Nocardia takedensis* Isolated from Koi (*Cyprinus carpio carpio*): A Novel Host Report

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ABSTRACT

Nocardia takedensis, defined as an environmental isolate and increasingly recognized as an opportunistic human pathogen, has no prior record in the literature regarding its isolation from fish. The aim of this study is to perform the first genome-based molecular characterization of the *N. takedensis* isolate NTD01, isolated from a koi carp (*Cyprinus carpio carpio*), a freshwater ornamental fish, in Türkiye, using Oxford Nanopore technology. The obtained long-read data were assembled *de novo* using the Flye algorithm, and taxonomic validation was performed through digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) analyses. The putative virulence-associated features and resistance profile of the isolate were examined at the genotypic level using the Comprehensive Antibiotic Resistance Database (CARD), Virulence Factors Database (VFDB), and the Antibiotics and Secondary Metabolite Analysis SHell (antiSMASH). The analyses revealed that the 8.96 Mb genome exhibited a nucleotide similarity (ANIb) of 99.18% and a dDDH value of 86.6% with the *N. takedensis* type strain. While four antimicrobial resistance genes associated with rifamycin and glycopeptide resistance were identified in the genome, no classical virulence factors were detected. In contrast, 33 biosynthetic gene clusters (BGCs) were identified, including nocobactin-like metallophore clusters involved in iron acquisition. Consequently, this study represents, to the best of our knowledge, the first report documenting the isolation and genome-based characterization of *N. takedensis* from koi fish. However, broader surveillance studies and experimental investigations are required to clarify the ecological distribution, pathogenic significance, and potential host association of this species in aquatic environments.

Keywords: *Cyprinus carpio carpio*, *Nocardia takedensis*, novel host, ornamental fish, whole-genome sequencing

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Öz: Koi (*Cyprinus carpio carpio*) Balığından İzole Edilen *Nocardia takedensis*'in Genom Temelli Karakterizasyonu: Yeni Konak Bildirimi

Çevresel bir izolat olarak tanımlanan ve zamanla fırsatçı bir insan patojeni olarak değerlendirilmeye başlanan *Nocardia takedensis*'in balıklardan izole edildiğine dair bugüne kadar literatürde herhangi bir bulguya rastlanmamıştır. Bu çalışmanın amacı, Türkiye'de tatlı su süs balığı olan koi (*Cyprinus carpio carpio*) balığından izole edilen *N. takedensis* NTD01 izolatının Oxford Nanopore teknolojisi kullanılarak genom temelli ilk moleküler karakterizasyonunu gerçekleştirmektir. Elde edilen uzun okuma verileri Flye algoritması ile de novo olarak birleştirilmiş, taksonomik doğrulama dijital DNA-DNA hibridizasyonu (dDDH) ve ortalama nükleotid kimliği (ANI) analizleriyle yapılmıştır. İzolatın olası virülansla ilişkili özellikleri ve direnç profili, kapsamlı Antibiyotik Direnç Veri Tabanı (CARD), Virülens Faktörleri Veri Tabanı (VFDB) ve Antibiyotik ve Sekonder Metabolit Analiz Aracı (antiSMASH) kullanılarak genotipik düzeyde incelenmiştir. Analizler sonucunda 8,96 Mb büyüklüğündeki genomun, *N. takedensis* tip suşu ile %86,6 dDDH ve %99,18 ortalama nükleotid kimliği temelinde hesaplanan nükleotid benzerliği (ANIb) gösterdiği tespit edilmiştir. Genomda rifamisin ve glikopeptid direnciyle ilişkili dört antimikrobiyal direnç geni saptanırken, klasik virülens faktörlerine rastlanmamıştır. Buna karşın, demir kazanımında rol oynayan nocobactin benzeri metallofor kümeleri de dahil olmak üzere 33 biyosentetik gen kümesi (BGC) belirlenmiştir. Sonuç olarak, bu çalışma, bilgimiz dahilinde, bir koi balığından *N. takedensis*'in izolasyonunu ve genom temelli karakterizasyonunu belgeleyen ilk rapordur. Bununla birlikte, bu türün sucul ortamlardaki ekolojik dağılımını, patojenik önemini ve potansiyel konak ilişkisini netleştirmek için daha kapsamlı sürveyans çalışmaları ve deneysel araştırmalar gereklidir.

Anahtar kelimeler: *Cyprinus carpio* carpio, *Nocardia takedensis*, yeni konak, süs balığı, tüm genom dizileme

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Introduction

Nocardia species, taxonomically classified within the phylum Actinomycetota, are Gram-positive, aerobic, filamentous bacteria widely distributed in soil, water, and decaying organic matter. Although traditionally regarded as environmental saprophytes, several *Nocardia* species are now recognized as opportunistic pathogens capable of causing nocardiosis in humans and animals, particularly in immunocompromised individuals or animals exposed to stress-related predisposing factors (Brown-Elliott et al. 2006).

Advances in molecular diagnostic methods have enabled the clearer detection of bacteria belonging to the genus *Nocardia* in both environmental and clinical samples in Türkiye. Türkiye's geographical and ecological diversity provides a highly favorable natural habitat for the survival of these microorganisms. During an environmental biodiversity study, *Nocardia goodfellowii* and *N. thraciensis* were isolated from soil samples collected in the Edirne region and were introduced to the scientific literature as novel species (Sazak et al. 2012). In addition, stress-tolerant strains of *N. niigatensis* have been isolated from boron-rich mining soils in the Bigadiç and Simav regions (Özdemir, 2026). The clinical significance of *Nocardia* infections is steadily increasing not only in environmental habitats but also in the field of animal health. For example, the identification of *N. puris* as a causative agent of mastitis in cattle in the province of Burdur was reported as the first documented case of this species in Türkiye (Yapicier et al. 2019).

In aquaculture, *N. seriolae* is considered the most commonly isolated and prevalent pathogen in farmed fish worldwide. However, other species, including *N. asteroides*, *N. salmonicida* and *N. crassostreae* have also been reported to cause infections in various fish species and aquatic organisms (Kudo et al. 1988; Maekawa et al. 2018; Wang et al. 2007). In Türkiye, *Nocardia* infections in aquaculture have begun to attract increasing attention, particularly with the development of marine fish farming. In the current literature, cases in Türkiye and the Mediterranean basin have been primarily associated with meagre (*Argyrosomus regius*) farming, and the causative agent has mostly been confirmed as *N. seriolae* using molecular methods (Elkesh et al. 2013; Yılmaz and Vural 2025).

Beyond this general description of *Nocardia* in the literature, the species *N. takedensis* was first isolated in 2005 from sediment collected from a moat surrounding the Takeda Shrine in Kofu, Japan, as well as from foaming activated sludge. The species name is also derived from the historical shrine from which it was first isolated (Yamamura et al. 2005). Initially identified solely as an environmental isolate, *N. takedensis* has gained clinical significance as an opportunistic pathogen in subsequent years, having been linked to primary cutaneous infections, lymphangitis, and respiratory tract infections in humans across various countries, including Spain, France, and South Korea (Betrán et al. 2009; Lee et al. 2017; Lotte et al. 2020).

The primary objective of this study was to perform genome-based molecular characterization of the *N. takedensis* NTD01 isolate, recovered from koi (*Cyprinus carpio carpio*), a freshwater aquarium fish in Türkiye, using Oxford Nanopore long-read technology. In this context, the study aimed to confirm the taxonomic placement of the isolate at the species level through phylogenomic approaches, including digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) analyses. Additionally, the genome was comprehensively annotated to characterize its structural and functional features, with particular emphasis on the identification of virulence factors, antimicrobial resistance genes, and biosynthetic gene clusters (BGCs). Based on these analyses, the study further aimed to provide preliminary genome-based insights into the ecological significance and possible host association of this isolate in ornamental aquaculture.

Materials and Methods

Bacterial strain and culture conditions

The *N. takedensis* NTD01 isolate was isolated incidentally from a koi fish in 2022 during a study investigating the distribution of mycobacterial species in aquarium fish in the province of Samsun (Hancı 2023). The fish did not exhibit characteristic gross lesions strongly suggestive of systemic nocardial infection at the time of sampling. For isolation, tissue samples obtained from the spleen, liver, and kidneys were pooled and homogenized. The resulting homogenate was subjected to a decontamination process (Shukla et al. 2013), and then 10 µL of the sample was inoculated onto Löwenstein–Jensen media (in 17×115 mm polycarbonate tubes, RTA Lab., Catalog No: 4007) and incubated at 30±1 ℃ (Zanoni et al. 2008). The pure cultures obtained were stored at -20 ℃ in Middlebrook 7H9 Broth containing 15% glycerol (Difco™ Middlebrook 7H9 Broth, BD 271310)

Whole genome sequencing (WGS)

Genomic DNA extraction from fresh bacterial colonies obtained from pure cultures was performed using a commercial extraction kit (PureLink® Genomic DNA Mini Kit, Invitrogen, Canada) based on the spin-column and filtration principle, in accordance with the manufacturer's instructions. In addition to the kit content, a 20 mL lysozyme lysis buffer containing 0.06 g of Tris-HCl, 0.15 g of

EDTA, 240 µL of Triton X-100, and 0.4 g of lysozyme was prepared for Gram-positive bacteria. DNA purity was assessed using the Multiskan™ GO Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA), while DNA concentration was determined using the Qubit™ 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

Whole-genome sequencing of the isolate was performed using long-read technology on an Oxford Nanopore PromethION 2 Solo instrument (Oxford Nanopore Technologies, UK). During the library preparation, the Native Barcoding Kit 24 V14 (SQK-NBD114-24; Oxford Nanopore Technologies) was used without DNA fragmentation to preserve genomic integrity and achieve maximum read length (Saticioglu et al. 2026). Raw reads were basecalled with Guppy (v6.1.7) in high-accuracy mode and processed for quality filtering and adapter trimming. Quality control and de novo genome assembly were performed using the Flye v. 2.9.1-b1780 algorithm (Kolmogorov et al. 2019) via the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) platform (Olson et al. 2023). The general statistical characterization and basic annotation of the genome were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) system in accordance with international GenBank standards (Tatusova et al. 2016).

Genome-based taxonomic assessment and phylogenetic analysis

Genome-based bioinformatics analyses were performed to determine the taxonomic position and phylogenetic relationships of the NTD01 isolate. dDDH values and phylogenomic tree reconstruction were carried out using the Type (Strain) Genome Server (TYGS) platform (Meier-Kolthoff and Göker 2019). ANI analyses were further conducted to confirm species-level classification with high resolution and to assess genomic relatedness to reference strains. In this context, both BLAST+-based ANI (ANIb) (Camacho et al. 2009) and MUMmer-based ANI (ANIm) (Kurtz et al. 2004) approaches were implemented via the JSpeciesWS web server. The resulting values were interpreted according to the widely accepted species delineation thresholds of ≥70% for dDDH and ≥95–96% for ANI (Chun et al. 2018).

Within the scope of the TYGS platform, intergenomic distances in pairwise comparisons were calculated using the Genome BLAST Distance Phylogeny (GBDP) method under the recommended parameters, and dDDH values, together with their confidence intervals, were obtained using GGDC 4.0 (Meier-Kolthoff et al. 2022; Meier-Kolthoff et al. 2013). Phylogenomic relationships were inferred from GBDP distance matrices using the FASTME v2.0 algorithm (Lefort et al. 2015), with branch support assessed through 100 bootstrap replicates. The resulting phylogenetic tree was subsequently rooted using the midpoint-rooting approach (Farris 1972; Kreft et al. 2017).

Identification of antimicrobial resistance genes, virulence and biosynthetic gene clusters

All genomic data obtained were subjected to comprehensive bioinformatics analyses to evaluate the pathogenic potential and antimicrobial resistance profile of the *N. takedensis* isolate NTD01 at the genotypic level. The Comprehensive Antibiotic Resistance Database (CARD) was screened using the Resistance Gene Identifier (RGI) tool to detect antimicrobial resistance genes (ARGs). To ensure high confidence in the analyses, 'Perfect' and 'Strict' hits criteria were applied, and threshold values of at least 80% sequence similarity and ≥80% coverage were used (Alcock et al. 2023).

To identify genes associated with virulence factors, the genome sequence was screened against the Virulence Factor Database (VFDB) full-length nucleotide dataset (Set B) using the BLASTn algorithm, and the analyses were conducted under default parameters (Liu et al. 2022). Biosynthetic gene clusters (BGCs) were identified using antiSMASH v8.0.4 software under 'relaxed' detection parameters (Blin et al. 2025).

Results

Whole genome sequencing (WGS)

The whole-genome sequencing data of the *N. takedensis* NTD01 isolate were assembled de novo using the Flye algorithm. The resulting draft genome comprised seven contigs with a total length of 8,968,604 bp. The genomic G+C content was calculated to be 69.18%. Genome annotation performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), based on the GeneMarkS-2+ algorithm, identified 7,939 protein-coding sequences (CDSs), 56 tRNA genes, 9 rRNA genes, and 271 partial CDSs. Genome quality assessment using CheckM estimated a completeness of 97.42% and a contamination level of 5.43%. The whole-genome sequencing project accession number was JBPZUD01, with assembly accession numbers GCA_051906215.1 and GCF_051906215.1. A summary of the genome assembly, annotation, and quality metrics is provided in Table 1.

Table 1. Genome assembly and annotation features of *N. takedensis* isolate NTD01.

Feature	Value
Number of contigs	7
Total genome length (bp)	8,968,604
G+C content (%)	69.18
Contig N50 (bp)	6,960,115
Contig L50	1
Protein-coding sequences (CDS)	7,939
Partial CDS	271
tRNA	56
rRNA	9
CheckM completeness (%)	97.42
CheckM contamination (%)	5.43
WGS project accession number	JBPZUD01
GenBank assembly accession number	GCA_051906215.1
RefSeq assembly accession number	GCF_051906215.1

Genome-based taxonomic assessment and phylogenetic analysis

Genome-based comparative analyses were conducted to determine the taxonomic position of the NTD01 isolate at the species level. dDDH results revealed that the isolate exhibited a high degree of genomic similarity to the type strain *N. takedensis* NBRC 100417. The obtained dDDH values (formulas d0, d4 and d6) were 86.6%, 96.8%, and 91.0%, respectively, all of which exceeded the 70% threshold accepted for prokaryotic species delineation (Table 2). In addition, ANI analyses revealed ANIb and ANIm values of 99.18% and 99.80%, respectively, between the NTD01 isolate and *N. takedensis* NBRC 100417 were obtained.

Table 2. Comparative genomics and dDDH values of isolate NTD01 against related type strains.

Query Strain	Target Strain	dDDH	C.I.	dDDH	C.I.	dDDH	C.I.	G+C content
		(d0, in %)	(d0, in %)	(d4, in %)	(d4, in %)	(d6, in %)	(d6, in %)	difference (in %)
NTD01	*Nocardia takedensis* NBRC 100417	86,6	[83.0 - 89.6]	96,8	[95.6 - 97.7]	91	[88.4 - 93.1]	0,36
NTD01	*Nocardia arizonensis* W9405	32	[28.6 - 35.6]	26,1	[23.7 - 28.6]	29,6	[26.7 - 32.7]	1,24
NTD01	*Nocardia arizonensis* NBRC 108935	32	[28.6 - 35.5]	26,1	[23.8 - 28.6]	29,5	[26.6 - 32.6]	1,23
NTD01	*Nocardia bovistercoris* NEAU-351T	27,2	[23.9 - 30.9]	24,8	[22.5 - 27.3]	25,6	[22.7 - 28.7]	1,01
NTD01	*Nocardia asiatica* NBRC 100129	21,1	[17.9 - 24.8]	24,3	[22.0 - 26.7]	20,6	[17.8 - 23.6]	0,74
NTD01	*Nocardia barduliensis* CECT 9924 T	21,4	[18.2 - 25.0]	24	[21.7 - 26.5]	20,8	[18.0 - 23.9]	0,67
NTD01	*Nocardia noduli* ncl1	26,6	[23.3 - 30.3]	23,7	[21.4 - 26.2]	24,9	[22.1 - 28.0]	2,15
NTD01	*Nocardia aurea* SYSU K10002	25	[21.6 - 28.6]	23,3	[21.0 - 25.8]	23,6	[20.7 - 26.7]	1,81
NTD01	*Nocardia amikacinitolerans* DSM 45539	21,6	[18.3 - 25.2]	23	[20.7 - 25.5]	20,8	[18.0 - 23.9]	0,69
NTD01	*Nocardia lijiangensis* NBRC 108240	21,5	[18.2 - 25.1]	23	[20.7 - 25.5]	20,7	[18.0 - 23.8]	0,69
NTD01	*Nocardia farcinica* NBRC 15532	22,4	[19.2 - 26.1]	22,9	[20.6 - 25.3]	21,5	[18.7 - 24.6]	1,5
NTD01	*Nocardia farcinica* NCTC11134	22,7	[19.4 - 26.3]	22,8	[20.5 - 25.3]	21,7	[18.9 - 24.7]	1,45
NTD01	*Nocardia farcinica* DSM 43257	22,6	[19.4 - 26.3]	22,8	[20.5 - 25.3]	21,6	[18.9 - 24.7]	1,5
NTD01	*Nocardia exalbida* NBRC 100660	21	[17.8 - 24.6]	22,4	[20.1 - 24.8]	20,3	[17.5 - 23.3]	0,56
NTD01	*Nocardia sputorum* IFM 12276	20,9	[17.7 - 24.5]	22,4	[20.1 - 24.8]	20,2	[17.4 - 23.2]	0,29

In the constructed phylogenomic tree, NTD01 isolate was clustered on the same branch as the *N. takedensis* NBRC 100417 type strain with a high bootstrap support value. The analysis clearly demonstrated that the isolate is distinctly separated from other *Nocardia* species, with *N. takedensis* representing its closest phylogenetic relative. Examination of interspecies relationships further revealed that *N. arizonensis*, *N. bovistercoris* and other related species formed distinct clades, confirming that the NTD01 isolate is phylogenetically separated from these groups (Figure 1).

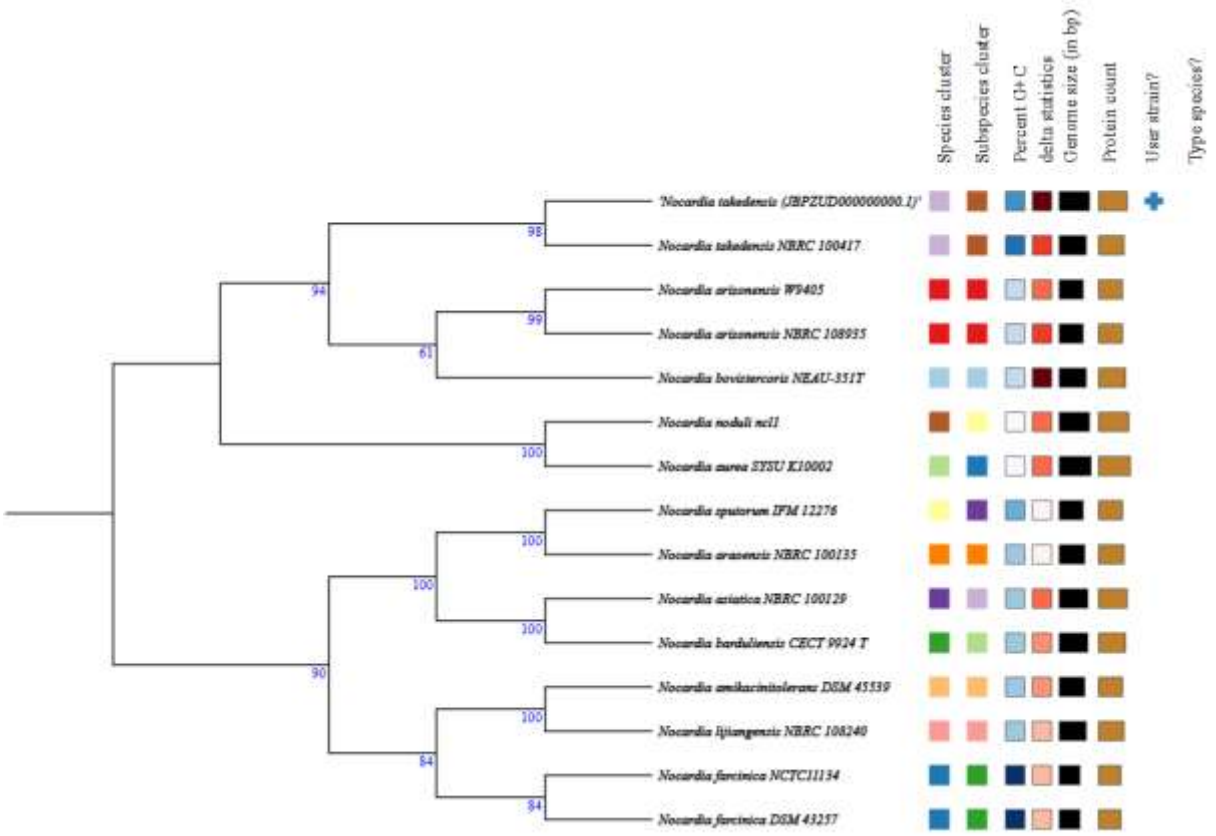

Figure 1. Phylogenomic tree based on whole-genome sequence analysis showing the relationship of isolate NTD01 to closely related type strains. The columns on the right provide additional genomic information: species and subspecies clusters are color-coded for classification purposes. Color gradients in the G+C content and delta statistics (δ values) columns represent relative variations for visual comparison. Genome size (bp) and protein count are represented by horizontal bars, where longer bars correspond to larger genomes or higher protein counts.

Identification of antimicrobial resistance genes, virulence and biosynthetic gene clusters

As a result of the RGI analysis performed against the CARD database, a total of four antimicrobial resistance genes meeting the 'Strict' criteria were identified in the genome of the NTD01 isolate, whereas no hits were found in the 'Perfect' or 'Loose' categories. The detected CARD/RGI hits corresponded to resistance-associated models related to rifamycin and glycopeptide antibiotic classes, (Table 3.). However, some identified hits, particularly *vanW* and *vanY*, exhibited relatively low similarity values, and therefore these findings should be interpreted cautiously as genomic predictions rather than confirmed phenotypic resistance determinants.

To identify virulence-associated genes, the genome sequence of isolate NTD01 was screened against the VFDB full-length nucleotide dataset (Set B) using BLASTn. Despite comparison with 34,500 virulence-associated sequences, no significant matches were identified in the isolate's genome. Accordingly, under the applied analytical parameters, no homologs of VFDB-annotated virulence genes were detected in the NTD01 genome. However, because VFDB is not specifically optimized for all *Nocardia*-associated pathogenicity determinants, the absence of VFDB hits should not be interpreted as definitive evidence of non-pathogenicity.

Table 3. Antimicrobial resistance genes detected in the NTD01 genome using

RGI Criteria	ARO Term	Detection Model	Antibiotic Class	Resistance Mechanism	Similarity %	Coverage (%)
Strict	*Mycobacterium tuberculosis rpoC* mutation (E1092D)	protein variant model	rifamycin	target alteration	88.01	100.08
Strict	*Nocardia farcinica rox*	protein homolog model	rifamycin	antibiotic inactivation	66.95	99.79
Strict	*vanW* (vanI cluster)	protein homolog model	glycopeptide	target alteration	35.50	148.79
Strict	*vanY* (vanB cluster)	protein homolog model	glycopeptide	target alteration	28.07	63.43

Table 4. Biosynthetic gene clusters identified in isolate NTD01 via antiSMASH analysis

antiSMASH Cluster No.	Biosynthetic Gene Cluster Type (BGC Type)	Similarity Level	Most Similar Known Cluster
4.1	T1PKS	-	-
4.2	T1PKS	-	-
4.3	NRPS, RiPP-like	Low	Corynecin I-III
4.4	Furan	-	-
4.5	Aminopolycarboxylic acid	Moderate	Ethylenediaminesuccinic acid hydroxyarginine
4.6	Terpene	High	Geosmin
4.7	NRPS	-	-
4.8	Terpene	-	-
4.9	Beta-lactone, NRPS, NI-	-	-
4.10	NRPS	-	-
4.11	NRPS	-	-
4.12	T1PKS, arylpolyene	Low	Kitacinnamycin A-F
4.13	T1PKS, T2PKS	Low	Apoptolidin
4.14	T1PKS	Low	Tetrocarcin A
4.15	Lanthipeptide class I	-	-
4.16	NRPS	-	-
4.17	Ectoine	High	Ectoine
4.18	Terpene, NRPS	Moderate	Isorenieratene
4.19	T1PKS	Low	Elaiophylin
4.20	NRPS	-	-
4.21	hglE-KS	-	-
4.22	Beta-lactone	-	-
4.23	Ranthipeptide	-	-
4.24	Terpene, T1PKS, arylpolyene	Low	Stenothricin
4.25	Terpene precursor	Low	Isorenieratene
4.26	NRPS-like, phosphonate	-	-
5.1	Butyrolactone	-	-
5.2	NRPS	-	-
5.3	NRPS	-	-
5.4	NRP-metallophore, NRPS	High	Nocobactin NA / nocobactin NA 10152B
5.5	Terpene, NRPS	-	-
5.6	T3PKS	-	-
5.7	NAPAA, PKS-like	Low	Caboxamycin

The genome sequence of the NTD01 isolate was analyzed using antiSMASH v8.0.4 software, and a total of 33 biosynthetic gene clusters were identified. These clusters primarily consist of type I polyketide synthase (T1PKS), type II polyketide synthase (T2PKS), and non-ribosomal peptide synthase (NRPS), along with clusters associated with terpenes, beta-lactones, lanthipeptides, ranthipeptides, phosphonates, and siderophores. Several identified BGCs also exhibited low to high levels of similarity to known biosynthetic pathways. A summary of the detected BGCs is provided in Table 4.

Discussion

Nocardia species are Gram-positive, aerobic actinobacteria commonly found in various environmental habitats such as soil, water, and decaying organic matter, and are capable of causing opportunistic infections (nocardiosis) in both humans and animals. (Brown-Elliott et al. 2006; McNeil and Brown 1994). These organisms are among the important bacterial agents responsible for systemic infections characterized by chronic granulomatous lesions in fish, leading to considerable economic losses in aquaculture (Wang et al. 2007). Among the species isolated from fish, the most frequently reported are *N. seriolae*, *N. salmonicida* and *N. asteroides* (Kudo et al. 1988; Maekawa et al. 2018; Wang et al. 2007). However, to the best of our knowledge, there are no previous reports of *N. takedensis* isolated from fish. *N. takedensis*, originally isolated from ditch sediment and activated sludge (Yamamura et al. 2005), has gained increasing significance in public health over the subsequent years and has been particularly associated with skin and soft tissue infections in humans (Brown-Elliott et al. 2006; Betrán et al. 2009; Lotte et al. 2020). In the present study, the isolation of *N. takedensis* from the internal organs of a koi carp represents a potentially novel host-associated record and expands the known ecological range of this species. This finding suggests that the bacterium is not restricted to environmental reservoirs but may also be associated with aquatic hosts. Considering the close interaction between ornamental fish and humans, this may represent a potential exposure pathway. However, because the current data are based on a single isolate and lack experimental infection evidence, this finding should not be interpreted as a definitive indicator of zoonotic risk. Instead, it highlights the need for broader epidemiological surveillance and experimental studies.

When analyzing high-G+C genomes such as those of *Nocardia* species, short-read sequencing technologies often result in highly fragmented assemblies. In contrast, the long-read data generated in this study using Oxford Nanopore technology were successfully assembled de novo with the Flye algorithm, yielding a high-quality draft genome of 8,968,604 bp distributed across only seven contigs. The reliability of the draft genome was validated through CheckM analysis, which indicated a high completeness of 97.42% and a moderate but acceptable contamination level of 5.43% (Parks et al. 2015). The genomic G+C content of the isolate was calculated to be 69.18%, which is consistent with the typically high G+C content observed in *Nocardia* species. Indeed, G+C content in *Nocardia* genomes has been reported to range from 65.5% to 72.0%, with an average value of 68.42% (Xu et al. 2021). Furthermore, genome sizes within the *Nocardia* genus have been reported to range from 5.04 Mb to 10.52 Mb, with the number of coding sequences (CDS) spanning from 4,626 to 9,617 (Xu et al. 2021). In the present study, the genome size of isolate NTD01 was determined to be 8.97 Mb, and a total of 7,939 CDS were identified. These findings indicate that the genomic features of isolate NTD01 are consistent with the general characteristics of the genus *Nocardia* and fall within the genomic range reported for this taxonomic group.

Genome-based phylogenetic analyses provide substantial advantages for reliable taxonomic classification, particularly in *Nocardia* species, where phenotypic similarities are high and species-level discrimination is often challenging. In this context, ANI analyses have been reported as a key tool for resolving taxonomic complexity within the genus *Nocardia*, enabling the re-evaluation of previously misidentified isolates. Indeed, Song et al. (2025) demonstrated that the application of ANI analyses led to the reclassification of numerous misidentified isolates and the recognition of 14 potential novel *Nocardia* species. In the present study, the widely accepted species delineation thresholds of ≥70% for dDDH and ≥95–96% for ANI (Chun et al. 2018) were used as the primary reference criteria for taxonomic evaluation. The high concordance of dDDH (86.6%), ANIb (99.18%), and ANIm (99.80%) values between isolate NTD01 and the reference strain *N. takedensis* NBRC 100417 provided strong evidence for accurate species-level identification and highlighted the high taxonomic resolution of the whole-genome-based approach. The findings demonstrate that high-quality whole-genome sequencing strategies provide a significant advantage in the precise identification of rare *Nocardia* species and support the routine application of WGS in epidemiological surveillance, zoonotic risk assessment, and clinical diagnostic workflows.

Nocardia species exhibit intrinsic resistance to numerous antibiotics due to their unique cellular

structure and biochemical properties. Furthermore, the addition of acquired resistance mechanisms, which can develop over time, to these innate resistance mechanisms further complicates the clinical management of *Nocardia* infections (Javadi et al. 2026). In the present study, the pathogenic potential of isolate NTD01 at the genetic level, together with the potential therapeutic challenges it may pose for both animal and public health, was comprehensively evaluated using specialized genomic databases. As a result of the CARD-RGI analysis, a total of four genes associated with resistance to rifamycin and glycopeptide groups of antibiotics were identified in the NTD01 genome (Table 3). Similarly, in a study evaluating *N. seriolae* strains isolated from fish, it was reported that nine isolates from Japan, North Korea, and China harbored two gene regions associated with rifamycin resistance (Islam et al. 2026). In another study, it was reported that the number of genes specifically associated with vancomycin resistance in *N. seriolae* genomes was approximately twofold more abundant in *N. seriolae* genomes compared with other *Nocardia* species (Han et al. 2019). These observations suggest that glycopeptide resistance may represent a notable characteristic of *Nocardia* species originating from aquatic environments and deserves further evaluation in relation to fish health and zoonotic risk. In addition to fish-associated isolates, resistance determinants related to rifamycin and vancomycin have also been identified in human-derived *Nocardia* isolates at the genomic level (Song et al. 2025). Moreover, the detection of high-level resistance to classical antimycobacterial agents such as rifamycin and ethambutol in human nocardiosis cases (Zhao et al. 2017) indicates that therapeutic options for these organisms may be limited. In this context, the detection of similar resistance determinants in the *N. takedensis* NTD01 strain isolated from koi carp represents an important finding that should be carefully considered not only for fish health management but also in terms of public health and potential zoonotic transmission. However, our findings represent genome-based predictions only and should not be interpreted as confirmation of phenotypic antimicrobial resistance.

A remarkable observation emerged when the pathogenicity mechanisms of isolate NTD01 were examined. In the analyses conducted using the VFDB database, no significant homologous genes belonging to classical virulence factors were identified. Although this finding may initially suggest a low pathogenic potential, previous studies have indicated that the pathogenicity of *Nocardia* species may not depend solely on classical virulence determinants but can also be strongly influenced by BGCs involved in the production of secondary metabolites (Männle et al. 2020).

Nocardia species are recognized for their remarkable capacity to produce secondary metabolites, harboring numerous BGCs within their genomes (Männle et al. 2020; Eripogu et al. 2025). In a large-scale genomic study, 263 *Nocardia* genomes were evaluated using antiSMASH analysis, identifying a total of 8,322 BGCs, with the number of clusters per genome ranging from 16 to 104. These clusters represented 46 different BGC classes, among which NRPS, T1PKS, terpene, and hybrid clusters were particularly predominant. Moreover, a significant positive correlation was reported between genome size and BGC abundance, indicating that larger genomes possess greater biosynthetic potential (Eripogu et al. 2025). In the present study, antiSMASH analysis identified 33 BGCs in the *N. takedensis* NTD01 genome, including multiple NRPS, T1PKS, terpene, lanthipeptide, ranthipeptide, β-lactone and hybrid clusters. Among these, the presence of nocobactin-like metallophore clusters with high similarity is particularly noteworthy, as these are considered important virulence-associated determinants in pathogenic *Nocardia* species, especially in relation to iron acquisition and host adaptation (Hoshino et al. 2011). Nocobactins have been reported to be structurally and biosynthetically closely related to mycobactins, which play a similar role in *Mycobacterium* species (Quadri et al. 1998). Mycobactins are known to be critical for the survival and replication of pathogenic mycobacteria, particularly *Mycobacterium tuberculosis*, within host macrophages and are regarded as key virulence factors (Reddy et al. 2013). Therefore, nocobactin-like systems may contribute to host adaptation or iron acquisition in *Nocardia*; however, their direct role in the pathogenicity of NTD01 remains to be experimentally demonstrated. Furthermore, the presence of numerous BGCs showing low sequence similarity or no correspondence to known clusters suggests that isolate NTD01 may have the capacity to produce novel and previously uncharacterized biologically active compounds. In particular, the lanthipeptide, ranthipeptide, and hybrid PKS-NRPS clusters are of special interest for the potential discovery of new antimicrobial agents.

Despite its increasing clinical significance for human health, data regarding the ecological role of this pathogen in aquatic ecosystems, its pathogenic effects on fish, and its potential zoonotic risks within the aquarium industry remain remarkably limited in the literature. To date, a review of the available literature reveals no evidence of *N. takedensis* isolation from either farmed or wild fish populations worldwide. Therefore, identifying the presence of previously unreported *Nocardia* species in aquatic

environments is of considerable importance for improving disease diagnosis and advancing our understanding of their epidemiology. To the best of our knowledge, this study represents the first scientific report documenting the isolation of *N. takedensis* from fish at the global level.

Conclusion

In conclusion, this study provides the first comprehensive whole-genome sequencing-based molecular characterization of the *N. takedensis* NTD01 strain isolated from koi carp, a freshwater ornamental fish in Türkiye. The high-resolution genomic data generated using Oxford Nanopore long-read technology strongly support the taxonomic identification of this rare pathogen, which is often difficult to distinguish using conventional phenotypic methods alone. Our findings suggest that ornamental freshwater fish may represent a previously unrecognized host-associated source of *N. takedensis*; however, broader surveillance studies are required to determine its distribution and epidemiological relevance in aquatic environments. The reference genomic data generated in this study are expected to provide a valuable foundation for future investigations and may contribute to improved epidemiological surveillance, risk assessment, and the development of effective management strategies for opportunistic pathogens with possible zoonotic significance in aquatic environments.

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