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Stenotrophomonas riyadhensis sp. nov., isolated from a hospital floor swab

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Abstract

During the analysis of a collection of *Pseudomonas* strains linked to an outbreak in an intensive care unit at King Faisal Specialist Hospital and Research Center in 2019, one isolate (CFS3442^T) was identified phenotypically as *Pseudomonas aeruginosa*. However, whole-genome sequencing revealed its true identity as a member of the genus *Stenotrophomonas*, distinct from both *P. aeruginosa* and *Stenotrophomonas maltophilia*. The isolate demonstrated: (i) a significant phylogenetic distance from *P. aeruginosa*; (ii) considerable genomic differences from several *S. maltophilia* reference strains and other *Stenotrophomonas* species; and (iii) unique phenotypic characteristics. Based on the combined geno- and phenotypic data, we propose that this isolate represents a novel species within the genus *Stenotrophomonas*, for which the name *Stenotrophomonas riyadhensis* sp. nov. is proposed. The type strain is CFS3442^T (=NCTC 14921^T=LMG 33162^T).

INTRODUCTION

The genus *Stenotrophomonas* comprises a group of Gram-negative bacteria that are known for their environmental versatility. They can be found in various habitats, including soil, water and even in association with plants [1–3]. Some species of this genus are opportunistic pathogens, with *Stenotrophomonas maltophilia* being the most clinically relevant due to its association with respiratory infections, especially in immunocompromised individuals, and its ability to acquire resistance to multiple antibiotics has made it a significant concern in clinical settings [4, 5]. In this study, we report the isolation and identification of a novel species of the genus *Stenotrophomonas*, which was initially misidentified as *Pseudomonas aeruginosa* (strain CFS3442^T) during an investigation of a potential outbreak in the intensive care unit (ICU) in the Department of Infection and Immunity of the King Faisal Specialist Hospital in Riyadh, Saudi Arabia. Environmental surface samples, including floor swabs, were systematically collected from various designated sites within the ICU. These samples were promptly transported to the laboratory under controlled conditions were aseptically inoculated onto blood agar and MacConkey agar. The plates were then incubated at 37°C for 18–24 h in an aerobic environment and, following incubation, distinct colonies were carefully selected based on their morphological characteristics and were subcultured onto fresh blood agar plates to obtain pure cultures. Initial identification based on phenotypic assays suggested these isolates were *P. aeruginosa* and included in a broader study [6]. Further biochemical identification using the API 20NE system (bioMérieux) revealed discrepancies in the initial identification.

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Abbreviations: BCCM, belgian coordinated collections of microorganisms; gDNA, genomic DNA; ICU, intensive care unit; MIC, minimal inhibitory concentrations; NCTC, national collection of type cultures; T, type strain.
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GenBank Whole Genome Sequence: JAHWBK000000000. BioSample: SAMN19868490. Assembly: GCA_025697965.1. BioProject: PRJNA739634. Nucleotide sequence: ID OQ852713.1 (16S ribosomal RNA gene sequence).
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Two supplementary figures and one supplementary table are available with the online version of this article.

WHOLE-GENOME SEQUENCING

Genomic DNA (gDNA) was extracted for short-read sequencing using the DNeasy UltraClean microbial kit (Qiagen) starting from pure isolated colonies resuspended in Luria–Bertani low-salt broth (Sigma-Aldrich) incubated for 18 h at 30°C. Purified DNA was quantified and quality assessed using a NanoDrop spectrophotometer (Thermo Fisher) and a Qubit fluorometer (Thermo Fisher). Genomic libraries were prepared using the NEBNext Ultra II FS (New England BioLabs) library preparation kit for Illumina. Adapter-ligated DNA fragments were size-selected from 700 to 800 bp using AMPure XP beads (Beckman Coulter) and final libraries were sequenced on the Illumina MiSeq platform using the reagent kit V3 (300 cycles paired-end) following the manufacturer's instructions. In addition gDNA was extracted and purified using the Wizard Genomic DNA kit (Promega) for long-read sequencing and library preparation was carried out using the Rapid Sequencing Kit (Oxford Nanopore Technologies) and loaded on a MinION (Oxford Nanopore Technologies) with a flongle flowcell.

DATA PROCESSING, ANNOTATION AND PHYLOGENETIC ANALYSIS

FastQ reads were quality assessed using FastQC (version 0.11.9) [7] and trimmed using FastP (version 0.20.1) [8] for Illumina or using Chopper (version 0.5.0) [9] for Oxford Nanopore outputs. *De novo* genome assemblies were generated using SPAdes (version 3.12.0) [10] for short reads and genome assembly for long reads was carried out using Flye (version 2.9.2) [11] obtaining 37 and one contig, respectively, with an average G+C content of 66.1 mol%. The resultant draft genome sequence was deposited in GenBank under the BioProject PRJNA739634 BioSample SAMN19868490 (sample name CFS3442^T).

The assembled genome was screened for antibiotic resistance-encoding genes using ABRicate version 1.0.1 [12] interrogating the Comprehensive Antibiotic Resistance Database [13]. Annotation was carried out with Prokka [14] (version 1.14.6), leading to the identification of 4510 coding sequences, five rRNA sequences, 74 tRNA sequences and one tmRNA sequence. The 16S ribosomal RNA gene sequence was retrieved and is available under the nucleotide sequence ID OQ852713.1.

Table 1. Overview of results for correlation coefficients (z-score) based on tetra correlation search of the reference database GenomesDB (JSpeciesWS)

In the table NCBI accession numbers of the hitlist sorted by highest correlation are presented.

Species	Strain	Z-Score	GenBank assembly no.
Stenotrophomonas maltophilia	ATCC 13637^T	0.99929	GCA_000742995.1
Stenotrophomonas maltophilia	NBRC 14161^T	0.99929	GCA_001591205.1
Stenotrophomonas maltophilia	MTCC 434^T	0.99925	GCA_000597745.1
Stenotrophomonas maltophilia	CGMCC 1.1788^T	0.99924	GCA_025617355.1
Stenotrophomonas maltophilia	NCTC10257^T	0.99916	GCA_900186865.1
Stenotrophomonas sepilia	SM16975^T	0.99897	GCA_003244875.1
Stenotrophomonas muris	DSM 28631^T	0.99896	GCA_024621935.1
Stenotrophomonas geniculata	JCM 13324^T	0.99884	GCA_001431625.1
Pseudomonas hibiscicola	ATCC 19867^T	0.99867	GCA_000382065.1
Stenotrophomonas pavanii	LMG 25348^T	0.99759	GCA_019704495.1
Stenotrophomonas pavanii	DSM 25135^T	0.99744	GCA_001431565.1
Stenotrophomonas indicatrix	WS40^T	0.99489	GCA_002750975.1
Stenotrophomonas lactitubi	M15^T	0.99467	GCA_900188015.1
Stenotrophomonas cyclobalanopsidis	TPQG1-4^T	0.98678	GCA_008710035.1
Stenotrophomonas nematodicola	CPCC 101271^T	0.97918	GCA_009467805.1
Stenotrophomonas rhizophila	DSM14405^T	0.97481	GCA_000661955.1
Stenotrophomonas lacuserhaii	K32^T	0.97108	GCA_014596295.1
Stenotrophomonas bentonitica	DSM 103927^T	0.97066	GCA_013185915.1
Stenotrophomonas chelatiphaga	DSM 21508^T	0.97030	GCA_001431535.1
Stenotrophomonas pennii	Sa5BUN4^T	0.96983	GCA_014836545.1

Table 2. Digital DNA–DNA Hybridization (dDDH) values and G+C content of strain CFS3442^T compared to genomic close type strains

Subject strain	GenBank assembly no.	dDDH (d0,%) [C.I.]*	dDDH (d4,%) [C.I.]*	dDDH (d6,%) [C.I.]*	G+C (mol%)*
S. maltophilia ATCC700475^T	GCA_013004645.1	67.4 [63.5–71.0]	47.3 [44.7–49.9]	64.3 [61.0–67.5]	0.14
S. maltophilia NBRC14161^T	GCA_000742995.1	65.9 [62.1–69.5]	45.7 [43.1–48.3]	62.5 [59.2–65.7]	0.01
S. geniculata JCM13324^T	GCA_001431625.1	66.4 [62.6–70.0]	45.6 [43.1–48.2]	62.9 [59.6–66.1]	0.01
S. muris DSM28631^T	GCA_024621935.1	68.7 [64.8–72.3]	45.2 [42.7–47.8]	64.6 [61.3–67.8]	0.51
P. hibiscicola ATCC19867^T	GCA_000382065.1	65.3 [61.5–69.0]	43.9 [41.3–46.4]	61.4 [58.2–64.6]	0.27
S. sepilia SM16975^T	GCA_003244875.1	62.2 [58.5–65.8]	43.7 [41.2–46.3]	58.8 [55.6–62.0]	0.28
S. pavanii DSM 25135^T	GCA_001431565.1	63.1 [59.4–66.7]	43.3 [40.8–45.9]	59.5 [56.3–62.7]	1.22
P. beteli LMG 978^T	GCA_001431665.1	66.8 [63.0–70.5]	41.3 [38.9–43.9]	61.7 [58.4–64.9]	0.66
S. cyclobalanopsidis TPQG1-4^T	GCA_008710035.1	50.9 [47.5–54.4]	34.1 [31.7–36.6]	46.5 [43.5–49.6]	0.98
S. indicatrix WS40^T	GCA_002750975.1	59.9 [56.2–63.4]	32.5 [30.1–35.0]	52.5 [49.4–55.5]	0.25

*G+C (mol%) indicates the difference in G+C content between the subject strain and strain CFS3442^T; C.I. is the confidence interval; d0, d4, and d6 refer to different formulae used to calculate dDDH values [17].

For comparative analysis, the assemblies were matched against the genomes of *S. maltophilia* (with NCBI assembly accession numbers: GCA_000742995.1, GCA_025617355.1, GCA_000597745.1, GCA_001591205.1 and GCA_900186865.1) and other *Stenotrophomonas* species (as detailed in Table 1). The selection was based on tetra nucleotide frequencies and correlation coefficients (z-score) [15] utilising the JSpeciesWS genome database [16] (Table 1).

The genome sequence data were analysed for a whole genome-based taxonomic analysis using the pipeline TYGS [17] and the pairwise comparisons among the set of genomes were conducted using GBDP for the phylogenomic inference [18]. Genomic relatedness between strain CFS3442^T and other genomically closer type strains, was determined by digital DNA–DNA hybridization (dDDH; Table 2) as previously described [19].

*S. maltophili*a ATCC700475^T (Hugh 1981 and *Pseudomonas maltophilia* ex Hugh and Ryschenkov 1960) had a dDDH value of 67.4%, suggesting a significant genomic similarity to strain CFS3442^T. Average nucleotide identity (ANI) values with strains with the ten highest dDDH values were calculated based on BLAST+ and MUMmer [20] and were also included in the calculation of pairwise tetra-correlation (Table 3).

A phylogenetic tree was reconstructed using parsnp (version 1.7.4) [21] based on core genome alignment of single nucleotide polymorphisms (Fig. 1).

A BLAST search using the 16S rRNA gene sequence of strain CFS3442^T revealed near-identical sequence similarities to *S. maltophilia* strains SJTL3^T and FZD2^T (GenBank numbers: CP029773.1 and CP080573.1), with identities of 1541/1541 and 1540/1541, respectively. High tetra values (>0.99) were observed for other species in addition to *S. maltophilia* (e.g. *S. sepilia*, *S. muris*, *S. geniculata*, *P. hibiscicola*, *S. pavanii*, *S. indicatrix* and *S. lactitubi*). These generally corresponded to high ANI values (>95%), suggesting a close genetic relationship among the genomes [22], However, the closest percentage values for both ANIb and ANIm for strain CFS3442^T were <92.00 and 93.00, respectively, when compared to any of the tested strains (Table 3); specifically, strain *S. maltophilia* ATCC 700475^T (GenBank assembly number GCA_013004645.1). These genomic characteristics, combined with the phylogenetic distance and inferred DDH <70% relative to any other reported species, support the rationale for assigning these isolates to a novel species within the genus *Stenotrophomonas*.

DESCRIPTION OF *STENOTROPHOMONAS RIYADHENSIS* SP. NOV.

Stenotrophomonas riyadhensis (ri.yadh.en'sis. N.L. fem. adj. riyadhensis, pertaining to Riyadh).

Table 3. Comparison of genomic relatedness metrics between CFS3442^T and other genomically related strains

The metrics include average nucleotide identity using BLAST (ANIb), average nucleotide identity using MUMmer (ANIm) and tetra nucleotide z-scores. Each strain is identified by its name and corresponding GenBank assembly number.

Subject strain	GenBank assembly no.	ANIb [%]	ANIm [%]	Tetra z-score
S. maltophilia ATCC700475^T	GCA_013004645.1	91.75	92.65	0.99835
S. maltophilia NBRC14161^T	GCA_000742995.1	91.24	92.36	0.99877
S. geniculata JCM13324^T	GCA_001431625.1	91.12	92.34	0.99839
S. muris DSM28631^T	GCA_024621935.1	91.10	92.3	0.99896
P. hibiscicola ATCC19867^T	GCA_000382065.1	90.45	91.91	0.99841
S. sepilia SM16975^T	GCA_003244875.1	90.50	91.85	0.99837
S. pavanii DSM 25135^T	GCA_001431565.1	90.50	91.68	0.99703
P. beteli LMG 978^T	GCA_001431665.1	89.89	91.25	0.99785
S. cyclobalanopsidis TPQG1-4^T	GCA_008710035.1	86.76	89.36	0.98649
S. indicatrix WS40^T	GCA_002750975.1	86.42	88.71	0.99491

Gram-negative, rod-shaped bacterium isolated from a floor swab of the ICU at King Faisal Specialist Hospital and Research Center. Initially identified as *Pseudomonas aeruginosa*, whole-genome sequencing later revealed its true classification within the genus *Stenotrophomonas*. Genomically, it is distinct from both *P. aeruginosa* and *S. maltophilia*. Notably, the strain lacks plasmids but possesses all components of the smeABC and smeDEF complexes, which are associated with the outer membrane multidrug efflux protein of *S. maltophilia*. Additionally, the strain carries the gene *aph(3')-IIc,* coding for a chromosomally

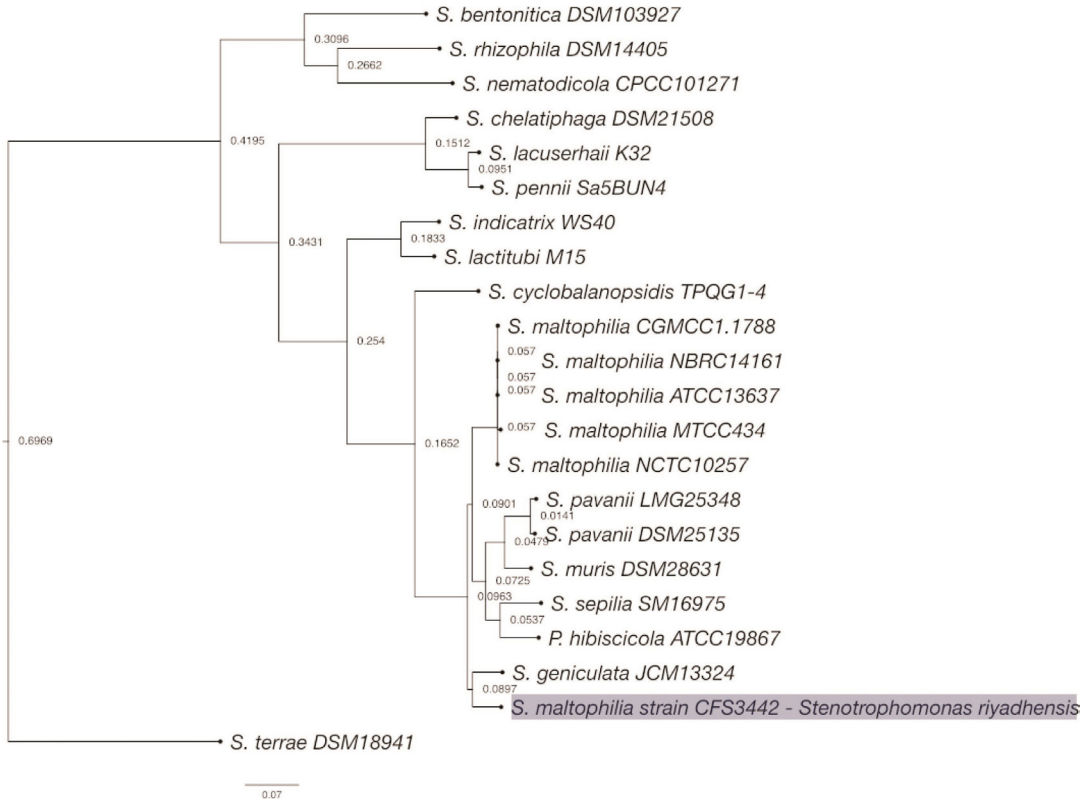

Fig. 1. Phylogenetic analysis of the strain identified as *Stenotrophomonas maltophilia* (strain CFS3442^T) and related species. The phylogenetic tree is reconstructed from core genome single nucleotide polymorphisms, showcasing the evolutionary relationship between strain CFS3442^T and close type strains of *Stenotrophomonas* species. In the analysis was included the strain *Stenotrophomonas terrae* DSM18941^T (GCA_001431465.1) as an outgroup.

encoded aminoglycoside phosphotransferase and *L1_beta-lactamase*, an Ambler class B metallo-β-lactamase with broad activity against beta-lactams. Phenotypic and biochemical characterizations were conducted using standard techniques, such as Gram staining, colony morphology assessment, and catalase and oxidase tests, as outlined in *Bergey's Manual of Determinative Bacteriology* [23]. The strain grows on Luria–Bertani low-salt agar (0.5% sodium chloride) and in trypticase soy broth, when incubated at 25, 30, 37 and 42°C aerobically as the optimal condition, and shows no growth at 4 and 50°C. The strain was also tested for growth in different media, including blood agar, eosin methylene blue agar, trypticase soy agar, Simmons citrate agar with 1% inositol and de Man–Rogosa–Sharpe agar, and incubated for 24h+24h at 25, 30, 37 and 4°C under both aerobic and anaerobic conditions (Table 4).

The sodium chloride ranges for growth were determined in Luria–Bertani medium supplemented with 0.5, 1.0, 2.0, 3.0, 4.0 or 5.0% (w/v) sodium chloride and a control with 0% (w/v) sodium chloride, and subjected to pH-tolerance tests at 30°C in trypticase soy broth. The strain showed optimal growth with 0.5–2.0% (w/v) sodium chloride and resistance at pH 4, 6 and 8, but it did not tolerate higher concentrations of salt (>4.00%) or pH 10, as detailed in Table 4. Further biochemical and physiological evaluations were performed using the API 20NE system (bioMérieux). The strain utilizes the following substrates as sole carbon sources for growth and assimilation: glucose, mannose, *N*-acetyl-glucosamine, maltose, capric acid and malate, while does not assimilate arabinose, mannose, potassium gluconate, adipic acid, trisodium citrate or phenylacetic acid. In the API 20NE system, the strain is positive for nitrate reduction, urease, *p*-nitrophenyl β-D-galactopyranoside, aesculin and gelatin hydrolysis, and is negative for indole production from tryptophan, glucose fermentation and arginine dihydrolase.

Strain CFS3442^T was subjected to mass spectrometry microbial identification using the VITEK MS system (bioMérieux), which employs matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) technology. The sample preparation involved direct colony transfer onto the MALDI-TOF plate followed by overlay with matrix solution. After air-drying, the plate was introduced into the mass spectrometer, where the bacterial proteins were ionized and their mass-to-charge ratio was measured. The resulting protein mass fingerprint was automatically compared to known spectra in the VITEK MS database and the analysis led to the identification of the strain as *S. maltophilia*. Chemotaxonomic analysis included the characterization of respiratory quinones, profiling of polar lipids and cellular fatty acids and these assays were carried out commercially (Leibniz-Institut DSMZ, Germany). The detected respiratory quinones (Q) were predominantly Q-8 (96.0%), with minor amounts of Q-7 (3.7%) and Q-9 (0.3%). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, aminolipid and lipid (Fig. S1, available in the online version of this article). The predominant fatty acids identified by GC-MS were $C_{15:0}$ iso (retention time, 7.234 min; area, 40.8117), $C_{15:0}$ anteiso (retention time, 7.356 min; area, 13.0507), $C_{14:0}$ (retention time, 6.378 min; area, 10.6538) and $C_{12:0}$ aldehyde (retention time, 3.319 min; area, 8.2406) (Table S1 and Fig. S2). The unique phenotypic characterization of strain CFS3442^T was compared through a comprehensive analysis, contrasting its traits with those of genomically related strains (Table 5). The comparative analysis demonstrates that while strain CFS3442^T shares several common phenotypic traits with its closest species, such as growth in a similar range of sodium chloride concentrations and resistance to various pH levels, it also exhibits unique properties. Strain CFS3442^T shows a distinct fatty acid profile and a different pattern of substrate assimilation, highlighting its unique metabolic capabilities. Additionally, the strain's resistance to higher salt concentrations and its inability to tolerate certain pH extremes further distinguish it from other species.

The complete phenotypic profile, as detailed in Table 5, underpins the classification of the strain as a novel species within the genus *Stenotrophomonas*.

Minimal inhibitory concentrations (MICs) were determined using Sensititre Gram-negative GN4F plates (Trek Diagnostic Systems) following the manufacturer's guidelines. The MIC results obtained were then interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints for *Enterobacteriaceae* [24]. The antibiotic susceptibility profile obtained highlighted the resistance of strain CFS3442^T to trimethoprim/sulphamethoxazole while it was found to be susceptible to aminoglycosides and tetracyclines, as previously described in *S. maltophilia*. When the genome of strain CFS3442^T, was queried for markers of antimicrobial resistance, aph(3′)-IIc was identified and which encodes for a chromosomally mediated aminoglycoside phosphotransferase known to confer resistance to aminoglycoside antibiotics. Additionally, the presence of the L1_beta-lactamase gene, an Ambler class B metallo-β-lactamase, suggests a broad resistance profile against beta-lactam antibiotics [25]. Despite the presence of these genes, phenotypic analysis confirmed the susceptibility of the strain to aminoglycosides, contradicting the typical resistance pattern conferred by the aph(3′)-IIc gene [26]. This sensitivity could be attributed to the regulation, expression levels or potential mutations in the corresponding open reading frame, thereby affecting its function. Conversely, strain strain CFS3442^T exhibited resistance to trimethoprim/sulphamethoxazole, a trait that is commonly observed in *S. maltophilia* and could be linked to other resistance mechanisms within the genome [27]. The MIC data provide phenotypic confirmation of the genotypic potential for resistance. These findings underscore the importance of performing both genomic and phenotypic analyses to fully understand the resistance potential of bacterial strains. The correlation between the genetic basis of resistance and the phenotypic resistance observed through MIC testing is essential for accurate antimicrobial therapy guidance, particularly for strains like CFS3442^T that exhibit an atypical resistance profile.

Table 4. Salt and pH tolerance and media growth profile of strain CFS3442^T

Test*	Media and condition	Temp. (°C)	Result growth 24 h	Result growth 48 h	Result growth 24 h anaerobic	Result growth 48 h anaerobic
Salt tolerance	LB 0% NaCl	25	–	–	ND	ND
	LB 0.5% NaCl	25	+	+	ND	ND
	LB 1.0% NaCl	25	+	+	ND	ND
	LB 2% NaCl	25	+	+	ND	ND
	LB 3% NaCl	25	–	+	ND	ND
	LB 4% NaCl	25	–	–	ND	ND
	LB 5% NaCl	25	–	–	ND	ND
	LB 0% NaCl	30	–	–	ND	ND
	LB 0.5% NaCl	30	+	+	ND	ND
	LB 1.0%NaCl	30	+	+	ND	ND
	LB 2% NaCl	30	+	+	ND	ND
	LB 3% NaCl	30	–	+	ND	ND
	LB 4% NaCl	30	–	–	ND	ND
	LB 5% NaCl	30	–	–	ND	ND
	LB 0% NaCl	42	–	–	ND	ND
	LB 0.5% NaCl	42	–	+	ND	ND
	LB 1.0% NaCl	42	–	+	ND	ND
	LB 2% NaCl	42	–	+	ND	ND
	LB 3% NaCl	42	–	+	ND	ND
	LB 4% NaCl	42	–	–	ND	ND
	LB 5% NaCl	42	–	–	ND	ND
	LB 0% NaCl	50	–	–	ND	ND
	LB 0.5% NaCl	50	–	–	ND	ND
	LB 1.0% NaCl	50	–	–	ND	ND
	LB 2% NaCl	50	–	–	ND	ND
	LB 3% NaCl	50	–	–	ND	ND
	LB 4% NaCl	50	–	–	ND	ND
	LB 5% NaCl	50	–	–	ND	ND
pH tolerance	TSB	30	+	+	ND	ND
	TSB pH 6	30	+	+	ND	ND
	TSB pH 8	30	+	+	ND	ND
	TSB pH 10	30	–	–	ND	ND
Growth media	Blood agar	25	+	+	–	–
	Eosin methylene blue agar	25	+	+	–	–
	Trypticase soy agar	25	+	+	–	–
	Simmons citrate agar with 1% inositol	25	–	–	–	–
	De Man–Rogosa	25	–	–	–	–
	Blood agar	30	+	+		
	Eosin methylene blue agar	30	+	+	–	–

Continued

Table 4. Continued

Test*	Media and condition	Temp. (°C)	Result growth 24 h	Result growth 48 h	Result growth 24 h anaerobic	Result growth 48 h anaerobic
	Trypticase soy agar	30	+	+	–	–
	Simmons citrate agar with 1% inositol	30	–	–	–	–
	De Man–Rogosa	30	–	–	–	–
	Blood agar	37	+	+		
	Eosin methylene blue agar	37	+	+	–	–
	Trypticase soy agar	37	+	+	–	–
	Simmons citrate agar with 1% inositol	37	–	–	–	–
	De Man–Rogosa	37	–	–	–	–
	Blood agar	4	–	–	–	–
	Eosin methylene blue agar	4	–	–	–	–
	Trypticase soy agar	4	–	–	–	–
	Simmons citrate agar with 1% inositol	4	–	–	–	–
	De Man–Rogosa	4	–	–	–	–

*+, Growth observed; –, no growth observed; ND, not determined or not applicable for the condition specified. LB=Luria-Bertani medium; TSB=Trypticase Soy Broth. The percentage next to NaCl denotes the concentration of sodium chloride in the medium.

Strain CFS3442^T has been deposited in two collections under the name *Stenotrophomonas riyadhensis* sp. nov.: the National Collection of Type Cultures (NCTC) with accession number NCTC 14921^T and the Belgian Coordinated Collections of Microorganisms (BCCM/LMG) under ID LMG 33162^T.

DISCUSSION AND IMPLICATIONS

The discovery of *S. riyadhensis* highlights the importance of accurate bacterial identification in clinical settings. Misidentification can lead to inappropriate treatment strategies, especially if bacteria are resistant to commonly used antibiotics. The fact that this bacterium was initially identified as *P. aeruginosa* underscores the challenges faced in bacterial taxonomy and the potential implications for patient care.

The genomic characteristics of strain CFS3442^T suggest that it has a significant genomic similarity to other strains, especially *S. maltophilia* ATCC 700475^T. However, its unique genomic features and phenotypic characteristics set it apart as a novel species.

The presence of genes associated with antibiotic resistance, such as the outer membrane multidrug efflux protein of *S. maltophilia*, is of particular concern. This highlights the potential for this bacterium to become a problematic pathogen in clinical settings, especially in environments such as ICUs where vulnerable patients are treated.

Given the increasing prevalence of antibiotic-resistant bacteria, it is crucial to continue monitoring and studying novel bacterial species. This will ensure that appropriate treatment strategies can be developed and implemented. The discovery of *S. riyadhensis* Methicillin-resistant Staphylococcus aureusserves as a reminder of the ever-evolving nature of microbial communities and the need for continuous research and surveillance.

RECOMMENDATIONS FOR FUTURE RESEARCH

The discovery of *S. riyadhensis* and its potential implications for public health, necessitates the development of proactive approaches towards implementing control strategies. The evolving landscape of microbial species in hospital settings necessitates vigilant monitoring and research. The following recommendations aim to address the challenges posed by this novel bacterium to ensure patient safety.

(1) Further studies to understand the pathogenicity of *S. riyadhensis* in humans. Understanding its pathogenicity is crucial. While it was isolated from a hospital environment, its potential impact on human health remains unclear a feature that has been highlighted for several other pathogens, including methicillin-resistant *Staphylococcus aureus* [28, 29], multi-drug resistant *Escherichia coli* [30, 31] and *Klebsiella* species [32, 33]. Determining its virulence factors, modes of transmission and

Table 5. Comparative phenotypic analysis of *Stenotrophomonas riyadhensis* CFS3442^T with related *Stenotrophomonas* species from the BacDive database [36]

Characteristic	*S. riyadhensis* CFS3442^T	*S. maltophilia* ATCC700475^T	*S. maltophilia* NBRC14161^T	*S. geniculata* JCM13324^T	*S. muris* DSM28631^T	*P. hibiscicola* ATCC19867^T	*S. sepilia* SM16975^T	*S. pavanii* DSM25135^T	*P. beteli* LMG978^T	*S. cyclobalanopsidis* TPQG1-4^T	*S. indicatrix* WS40^T
pH tolerance	4.0–8.0	NA	NA	NA	NA	NA	NA	0.5–12.0	NA	4.0–10.0	6.0–9.0
Optimal NaCl range for growth %(w/v)	0.5–2.0	0–4.0	0–4.5	0–4.0	NA	NA	NA	0.7–3.0	NA	1.0–3.0	NA
Growth temperature range (°C)	25.0–42.0	10.0–37.0	25–41	20.0–37.0	NA	NA	NA	20.0–37.0	NA	4.0–37.0	10.0–37.0
Growth temperature optimal (°C)	30.0	NA	NA	NA	37.0	28.0	NA	28.5	28.0	30.0	NA
Polar lipids†	DPG, PE, PG, AL, L	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fatty acid profile (representing values >4.0%)	$C_{15:0}$ iso (16.4%), $C_{15:0}$ anteiso (5.3%) $C_{14:0}$ (4.3%)	NA	NA	$C_{15:0}$ iso (50.3%), $C_{16:1}\omega7c$ (12.4%), $C_{17:1}\omega9c$ iso (5.6%)	NA	NA	NA	NA	NA	NA	$C_{15:0}$ iso (54.0%), $C_{15:0}$ anteiso (32.5%), $C_{11:0}$ iso (4.2%)
Positive API 20NE results‡	URE, PNPG, ESC, GEL, GLUa, NAG, MAL, CAP	ESC, NAG, MAL, GLU, MNE*	ESC, PNPG, GEL, GLU, MNE, NAG, MAL, MLT, CIT	ESC, GEL, PNPG, GLUa, MNE, NAG, MAL, MLT, CIT	ESC, PNPG, GLUa, MNE, NAG, MAL, MLT, CIT	ESC, GEL, PNPG, GLUa, MNE, NAG, MAL, CAP, MLT, CIT	NA	ESC, GEL, PNPG, GLUa, MNE, NAG, MAL, MLT, CIT	ESC, GEL, PNPG, GLUa, MNE, NAG, MAL, CAP, MLT, CIT	NA	ESC, GEL, PNPG, GLUa, MNE, NAG, MAL, MLT, CIT
Negative API 20NE results‡	TRP, GLUf, ADH, ARA, MNE, GNT, ADI, CIT, PAC	ARA*	TRP, GLUf, ADH, URE, ARA, MAN, GNT, CAP, ADI, PAC	TRP, GLUf, ADH, URE, ARA, MAN, GNT, CAP, ADI, PAC	TRP, GLUf, ADH, URE, GEL ARA, MAN, GNT, CAP, ADI, PAC	TRP, GLUf, ADH, URE, ARA, MAN, GNT, ADI, PAC	NA	TRP, GLUf, ADH, URE, ARA, MAN, GNT, CAP, ADI, PAC	TRP, GLUf, ADH, URE, ARA, MAN, GNT, ADI, PAC	NA	TRP, GLUf, ADH, URE, ARA, MAN, GNT, CAP, ADI, PAC

*Only some results available and data were estrapolated from API Biotype100 and API ZYM tests.
†DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, aminolipid; L, lipid.
‡API 20NE tests included: URE, urease; PNPG, *β*-galactosidase (*para*-nitrophenyl-*β*-D-galactopyranoside); ESC, aesculin hydrolysis; GEL, gelatinase; GLUa, glucose assimilation; NAG, *N*-acetyl-glucosamine assimilation; MAL, maltose assimilation; CAP, capric acid assimilation; TRP, indole production from tryptophan (L-tryptophan); GLUf, D-glucose fermentation; ADH, arginine dihydrolase (L-arginine); ARA, arabinose assimilation; MNE, mannose assimilation; GNT, gluconate assimilation; ADI, adipic acid assimilation; CIT, citrate assimilation; PAC, phenylacetic acid assimilation.
NA, missing values (not available).

potential to cause disease will provide insights into its clinical significance and guide healthcare professionals in managing potential infections.

(2) Research into potential treatment strategies, given its antibiotic resistance profile. The antibiotic resistance profile of *S. riyadhensis*, as indicated, suggests that it might pose challenges for treatment. Traditional antibiotics might be ineffective, necessitating research into alternative therapeutic approaches [28]. Exploring novel antimicrobial agents, phage therapy or combination therapies could be pivotal in combating infections caused by this bacterium.

(3) Continuous monitoring of hospital environments to detect and study the prevalence of such novel bacterial species. Hospital environments are hotspots for the emergence of novel and potentially pathogenic bacterial species, given the confluence of various microbial agents and antibiotic use [34, 35].

Continuous surveillance is essential not only to detect such species early but also to prevent potential outbreaks. Regular monitoring, combined with genomic sequencing, can provide a comprehensive understanding of the microbial dynamics in healthcare settings and inform infection control measures. Embracing these recommendations will strengthen our defences against emerging microbial threats, ensuring the well-being of patients and the broader community.

CONCLUSION

The identification of *S. riyadhensis* extends our understanding of the genus *Stenotrophomonas* and its potential implications in clinical settings. Continued research into this bacterium and others like it will be essential to ensure effective treatment strategies to counter ever-increasing antibiotic resistance.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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