Infection and Drug Resistance

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ORIGINAL RESEARCH

Identification of OqxB Efflux Pump and Tigecycline Resistance Gene Cluster *tmexC3D2-toprJ3* in Multidrug-Resistant *Pseudomonas Stutzeri* Isolate G3

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Purpose: To identify antibiotic resistance genes (ARGs) and understand the molecular basis of multidrug resistance in *P. stutzeri* isolate G3.

Methods: Whole-genome sequencing of isolate G3 was conducted at 30X coverage using Illumina NovaSeq 6000. Reads were trimmed using Trimmomatic and assessed using a combination of scripts that incorporated Samtools, BedTools, and bwa-mem. *De novo* assembly was performed using SPAdes, and assembly metrics were evaluated using QUAST. The assembled genome was uploaded to a Type Strain Genome Server (TYGS) for taxonomic identification. Genome annotation was performed using the KBase and Proksee software using PROKKA. ARGs were identified using the Comprehensive Antibiotic Resistance Database (CARD).

Results: *P. stutzeri* isolate G3 demonstrated resistance to most antibiotics tested, including meropenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and tetracycline (30 µg). The ARGs identified were *PmpM, AdeF, rsmA, vgb(A), BcI, cipA, OCH-2*, and *tet(45)*. A tigecycline-resistant gene cluster, *tmexC3D2-toprJ3*, was found in NODE_84, while the *oqxB* gene, encoding a resistance-nodulation-division (RND) efflux pump, was in NODE_309. Phylogenetic analysis showed *OqxB* clustered with *Pseudomonas* species, distinct from *Klebsiella* and *Enterobacter*. Comparative analysis of *oqxB* revealed *P. stutzeri* isolate G3 shared 78–100% identity with *Pseudomonas aeruginosa* strain 1334/14 in key components of the multidrug efflux system, including the transcriptional regulator *MexT*, periplasmic adaptor subunit *MexE*, and permease subunit *MexF*.

Conclusion: Our findings offer new insights into the reservoir of ARGs in the draft genome of *Pseudomonas stutzeri* isolate G3, including the *tmexC3D2-toprJ3* and *oqxB* genes, highlighting its genomic plasticity and public health significance. This adaptability enables *P. stutzeri* to thrive in clinical environments, despite its natural habitat association. This study advances our understanding of the molecular mechanisms driving resistance in *P. stutzeri* and offers valuable insights to inform strategies for combating the spread of antimicrobial resistance in clinical and environmental settings.

Keywords: *Pseudomonas stutzeri*, OqxB RND efflux pump, *tmexC3D2-toprJ3*, antibiotic resistance genes, bacterial genomics, whole-genome sequencing

Introduction

The release of antibiotic-containing wastes into water bodies drives the development of antibiotic resistance in environmental species such as *Pseudomonas stutzeri* with the potential to serve as a reservoir for multidrug resistance.[1] This pollution exposes communities that rely on surface water for recreation, tourism, irrigation, and drinking, to infections caused by drug-resistant pathogens. Vulnerable populations, including the elderly and immunocompromised individuals, are at greater risk of developing serious infections, highlighting their epidemiological significance.

P. stutzeri is ubiquitous in clinical settings and usually originates from the environment.[2] This opportunistic pathogen causes serious infections and death in patients with predisposing risk factors such as immunosuppression, previous surgery,

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and underlying illnesses.[2–4] Infections associated with *P. stutzeri* include bacteraemia, septicaemia, osteomyelitis, arthritis, endocarditis, endophthalmitis, panophthalmitis, meningitis, pneumonia, empyema, peritonitis, and ventriculitis.[1–4]

P. stutzeri exhibits resistance to multiple antibiotics, with antibiotic resistance genes (ARGs) located on chromosomes or mobile genetic elements such as plasmids.[5,6] For example, plasmid pT75-VIM harbours multiple ARGs, including the tigecycline resistance gene cluster *tmexCD1-toprJ1*.[6] Resistance mechanisms involve alterations in outer membrane proteins and lipopolysaccharide profiles as well as the production of β-lactamases that hydrolyse natural and semisynthetic penicillins, broad-spectrum cephalosporins, and monobactams.[2]

Efflux pumps also play a role in multidrug resistance of *P. stutzeri*. For example, the TbtABM efflux pump identified in tributyltin (TBT)-resistant *P. stutzeri* confers resistance to n-hexane, nalidixic acid, chloramphenicol, and sulfamethoxazole.[7] TbtABM belongs to the resistance-nodulation-division (RND) family, which includes other efflux pumps such as OqxB.[7–9] OqxB has been associated with resistance to various antimicrobial agents, including quinolones, fluoroquinolones, nitrofurantoin, quinoxalines, tigecycline, chloramphenicol, detergents, and disinfectants.[8,10–13]

Genes encoding the OqxB efflux pump are located on chromosomes and/or plasmids of some Gram-negative bacterial species, and are widespread in the environment, including hospital wastewater,[8] Mobile genetic elements, such as IS26 and plasmids, facilitate the spread of the *oqxB* gene from species like *K. pneumoniae, E. coli*, and *E. cloacae* to other Gram-negative species, including *Salmonella* spp.[8,10,14] The horizontal transfer and overexpression of *OqxB* contribute to multidrug resistance, posing a significant public health risk. For example, in *K. pneumoniae, OqxB* overexpression is a key factor in drug resistance among clinical isolates.[9]

P. stutzeri is a ubiquitous and versatile bacterium, largely due to its ability to acquire genes, such as *oqxB*, from its environment.[2] However, to our knowledge, there are currently no reports of OqxB efflux pumps in this species. While resistance to fluoroquinolones in *P. stutzeri* has been associated with the overexpression of efflux pumps like TbtABM,[15] OqxB has been implicated in fluoroquinolones extrusion in other species like *E.coli*.[12,13] Its role in contributing to multidrug resistance in *P. stutzeri r*emains poorly understood.

This study aimed to identify ARGs and elucidate the molecular basis of multidrug resistance in *P. stutzeri*. We identified several ARGs including a tigecycline-resistant gene cluster, *tmexC3D2-toprJ3*, and the *oqxB* gene, which encodes a resistance-nodulation-division (RND) efflux pump. Notably, this study is the first to report the potential involvement of the OqxB efflux pump in *P. stutzeri*. These findings provide new insights into the molecular mechanisms underlying resistance and may inform strategies to mitigate its spread in clinical and environmental contexts.

Material and Methods

Sample Collection and Bacteria Culture

The multidrug-resistant bacteria, isolate G3, was isolated from surface water collected from Gunwade Lake on 01/11/2022 (Figure 1). Gunwade Lake, located in Ferry Meadows, Peterborough's public-access nature park, is the primary hub for water activities. It features a scout camp, a sailing club, windsurfing, a canoe hire, swan pedal boats, and fishing spots on the southern side of the lake. A large café with a terrace overlooking the lake provides a relaxed spot for visitors.

The area is designed for easy accessibility, with car parks, tarmac paths, and boardwalks catering to the diverse mobility requirements. In the summer afternoons, Gunwade Lake became a bustling spot for locals.

One litre of surface-level water was collected in sterile glass jars with secure lids. The water temperature at the time of sampling was 13°C °C and the pH was 8. Water samples were transferred to ice boxes for transportation to the laboratory. They were cultured on MacConkey agar and nutrient agar for the total Gram-negative (TGN) bacterial count and Heterotrophic Plate Count (THC), respectively. Agar plates were inoculated with 100 µL of water samples from undiluted (10°) to 10^{-5} dilutions, and incubated at room temperature for 48 h. Colonies were counted and discrete colonies were picked and subcultured. Pure cultures were obtained by repeated subculturing using the streak-plating method. Gram staining, microscopic examination, oxidase testing, and morphological characterisation of the isolate were performed.

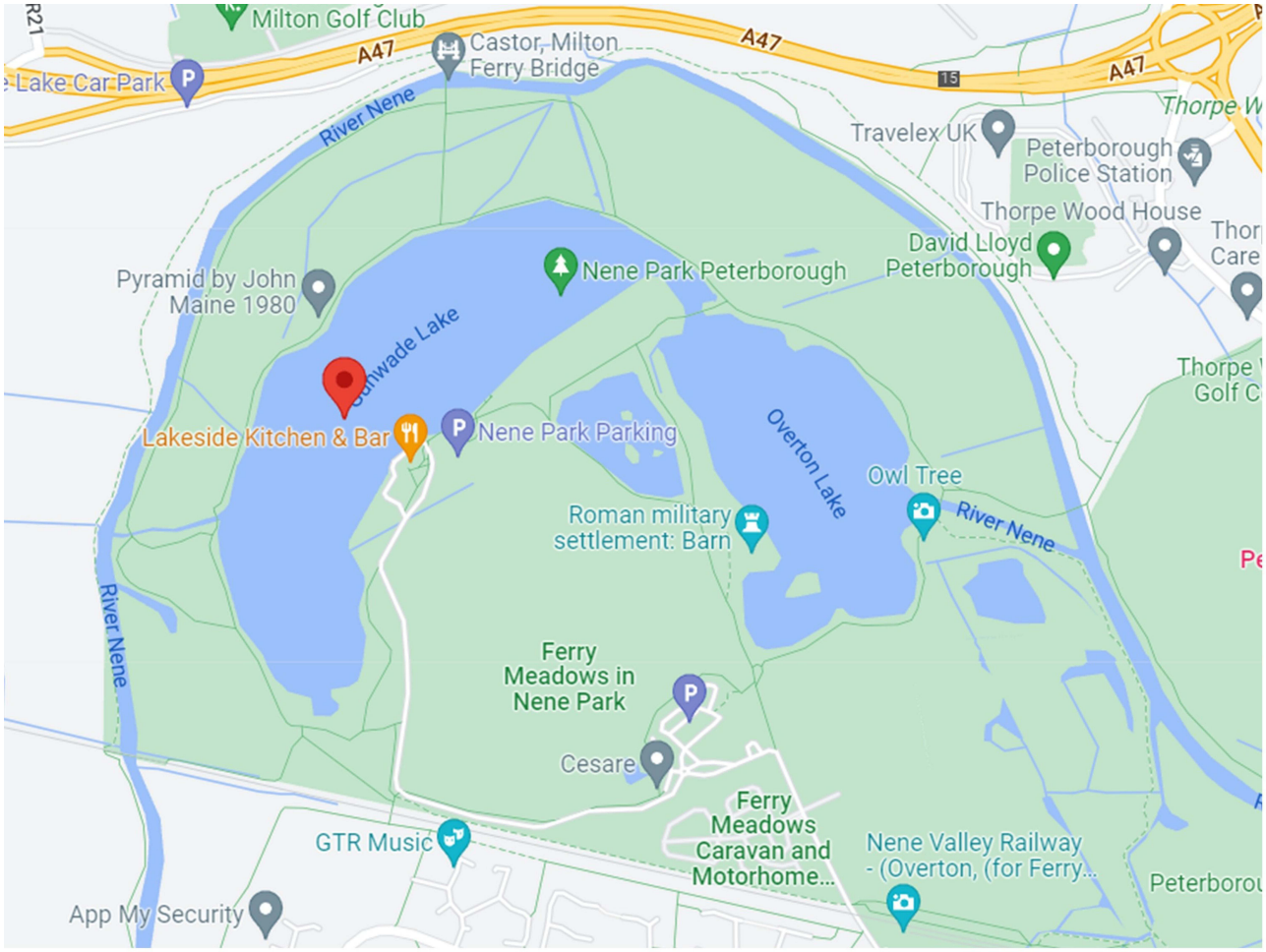

Figure 1 Google map showing Gunwade Lake indicated by the red pin where the water samples were collected.

Antibiotic Susceptibility Testing by Kirby Bauer Method

Antibiotic susceptibility testing was performed using the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.[16] Briefly, a lawn of pure bacterial culture was cultivated on Mueller-Hinton agar (MHA) plates with a maximum of 4 antibiotic discs per plate and incubated at 37°C for 24 h. The following antibiotics were tested: ciprofloxacin (5 µg), vancomycin (5 µg), trimethoprim (5 µg), meropenem (10 µg), gentamicin (10 µg), ampicillin (10 µg), erythromycin (10 µg), amoxicillin (10 µg), cefpodoxime (10 µg), streptomycin (10 µg), doxycycline (30 µg), tetracycline (30 µg), chloramphenicol (30 µg) and teicoplanin (30 µg). Guidelines published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI) were used to interpret the zone.[17,18]

Whole Genome Sequencing and Genome Assembly

Isolate G3 was prepared and submitted to MicrobesNG (Birmingham, UK) for DNA extraction, whole-genome sequencing, and bioinformatics analyses. The bacterial isolate (isolate G3) was prepared as described in the protocol for preparing strains for MicrobesNG in an inactivation buffer. The isolate was submitted to a strain submission tube containing a DNA/RNA shield (DRS) (Zymo Research, USA) provided by MicrobesNG.[19] Briefly, G3 cells were grown on nutrient agar and incubated at 37°C °C for 24 h. The bacterial culture was transferred into a sterile test tube containing 5 mL of 1 × PBS buffer using a sterile inoculating loop. As required, we measured the optical density (OD600nm) of the resuspended cells, which was 0.6 (below 1.0). The resuspended cells were concentrated by centrifugation at 2000rpm for 10 min in a 1.5mL tube, washed with 1mL PBS, and pelleted again. The final pellet was resuspended in 1x DRS buffer (0.5 mL) in a barcoded 2 mL

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screw cap tube provided by MicrobesNG, packaged, and sent off. The Genome of isolate G3 was sequenced at 30X coverage on an Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) using the 250 bp paired[20,21] -end protocol of MicrobesNG.[20] The reads were adapter-trimmed using Trimmomatic version 030[21] with a sliding window quality cutoff of Q15, and the quality was assessed using scripts combined with Samtools, BedTools, and bwa-mem. De novo assembly was performed on the samples using SPAdes version 3.7,[22] and the assembly metrics were calculated using QUAST.[23]

Genome Annotation and Identification of Antibiotic Resistance Genes (ARG) and Mobile Genetic Elements (MGEs)

The assembled genome was uploaded to the Type Strain Genome Server (TYGS)[24] for taxonomic identification. Identification was confirmed by phylogenetic analysis of the 16SrRNA gene of *Pseudomonas* species in MEGA11.[25] The genome was annotated with KBase[26] and Proksee[27] using PROKKA annotation pipeline.[28] In silico identification of antibiotic resistance genes (ARGs) and mobile genetic elements in the genome was performed using Proksee (https://proksee.ca), Comprehensive antibiotic resistance databases (https://card.mcmaster.ca) and ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder). Mobile genetic elements were identified using MobileOG-DB.[29] Easyfig 2.25, was used to produce the genetic context figure.[30]

Phylogenetic Analysis

Phylogenetic analysis was conducted in MEGA11[25] using the neighbour-joining method.[31] The phylogenetic tree produced was tested using a bootstrap test (1000 replicates) and is shown next to the branches.[32] Evolutionary distances were computed using the Maximum Composite Likelihood method.[33] The type strains used in the analyses were downloaded from the LPSN (https://www.bacterio.net/).[34] Genomes and *oqxB* gene files were downloaded from the NCBI database.

Results

Identification of Isolate G3

Gram-negative oxidase-positive isolate G3 was isolated from water samples from Gunwade Lake (Figure 1). Microbiological assessment of the water sample yielded a heterotrophic plate count (HPC) of 5.60×10^6 cfu/mL and a total Gram-negative count (TGNC) of 4.96×10^6 cfu/mL. The assembled genome of isolate G3 was submitted to TYGS and identified as *Pseudomonas stutzeri*, hereafter referred to as *Pseudomonas stutzeri* isolate G3. Phylogenetic analyses of the 16S rRNA gene from this isolate and other *Pseudomonas* species confirmed that isolate G3 was related to other *P. stutzeri* strains (Figure 2). The assembled genome size of strain G3 was 9,794,128 bp comprising 9396 coding and 90 non-coding genes. There were 5289 contigs, N50 = 2498, L50 = 847. Data for this project have been deposited in NCBI under BioProject accession number PRJNA1028652.

Antibiotic Susceptibility Profile of Pseudomonas Stutzeri Isolate G3

P. stutzeri isolate G3 demonstrated resistance to most antibiotics tested, including meropenem (10 µg) (Table 1, Tables S1 and S2). It was also resistant to ciprofloxacin (5 µg), gentamicin (10 µg), and tetracycline (30 µg) (Table 1). The average zone diameters of diverse antibiotic classes at 10 µg disk content (Table S1) ranged from 0.0 mm for meropenem to 28 ± 7 mm (n = 5) for ciprofloxacin. Larger inhibition zones of 23 ± 6 (n = 5) and 28 ± 7 mm (n = 5) were observed for gentamicin (10 µg) and ciprofloxacin (10 µg), respectively.

In Silico Identification of Antibiotic-Resistant Genes in the Draft Genome of Pseudomonas Stutzeri Isolate G3

The draft genome of *P. stutzeri* isolate G3 contained several antibiotic resistance genes (Figure 3 and Table 2). A tigecycline-resistant gene cluster, *tmexC3D2-toprJ3*, was detected on NODE_84 of the draft genome (Table 2). *tet (45)*, which confers resistance to doxycycline and tetracycline, was also identified in NODE 3096 (Table 2). Other ARGs identified in *P. stutzeri* isolate G3 were *PmpM, AdeF, rsmA*, vgb(A), *BcI, cipA* and *OCH*-2, conferring resistance to diverse antimicrobial agents (Table 2). The identified genes conferred antibiotic resistance to a wide range of antibiotic

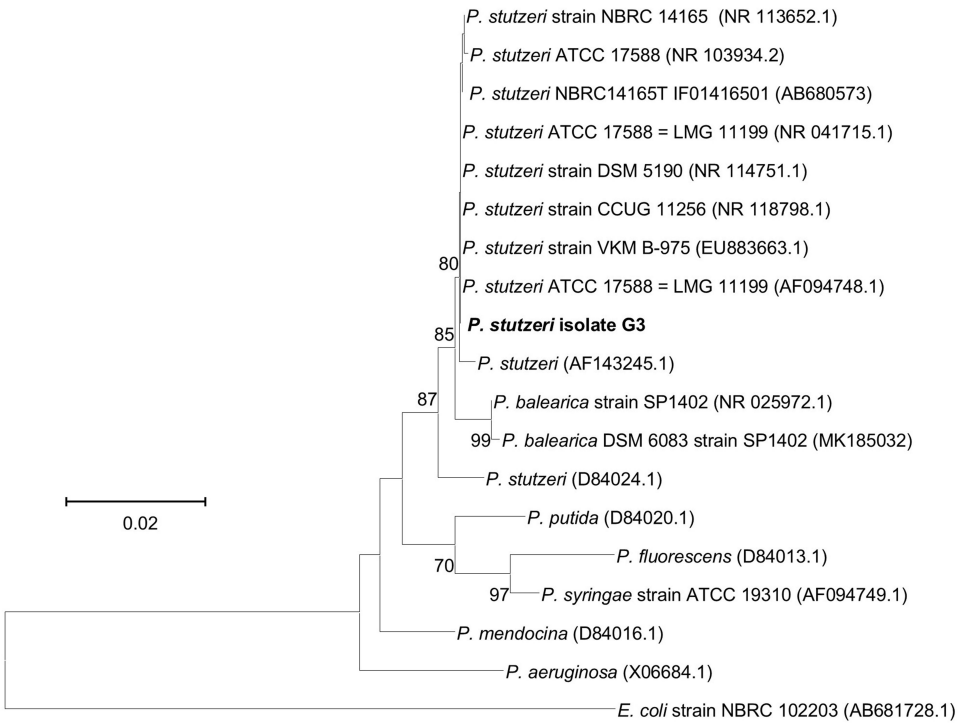

Figure 2 Phylogenetic relationship of *Pseudomonas stutzeri* isolate G3 and other *Pseudomonas* species based on *16S rRNA* gene analyses. The evolutionary history was inferred using the Neighbor-Joining method.[31] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.[32] The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method[33] and are in the units of the number of base substitutions per site. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 848 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.[25]

classes, including those tested in the laboratory namely Fluoroquinolones, Carbapenems, Aminoglycosides, and Tetracyclines (Table 1).

The gene *oqxB* encoding the resistance-nodulation-division (RND) efflux pump, was found in NODE_309 (Table 2). *P.stutzeri* isolate G3 and *P. aeruginosa* strain 1334/14 had a similar (78–100% identity) multidrug efflux system transcriptional regulator MexT, multidrug efflux RND transporter periplasmic adaptor subunit MexE, and multidrug efflux RND transporter permease subunit MexF (Figure 4). However, the arrangement of *oqxB* gene components differed between the two species (Figure 4). Phylogenetically, *OqxB* clustered with other *Pseudomonas* species but differed from *Klebsiella* and *Enterobacter* species (Figure 5).

Table 1 Antibiotic Susceptibility Test Result of Pseudomonas Stutzeri Isolate G3

Antimicrobial Agent	Class	Disk Content	Zone Diameter (Nearest Whole mm)	Antimicrobial Agent
Ciprofloxacin	Fluoroquinolones	5µg	10	Resistant
Meropenem	Carbapenems	10µg	0	Resistant
Gentamicin	Aminoglycosides	10µg	7	Resistant
Tetracycline	Tetracyclines	30µg	3	Resistant

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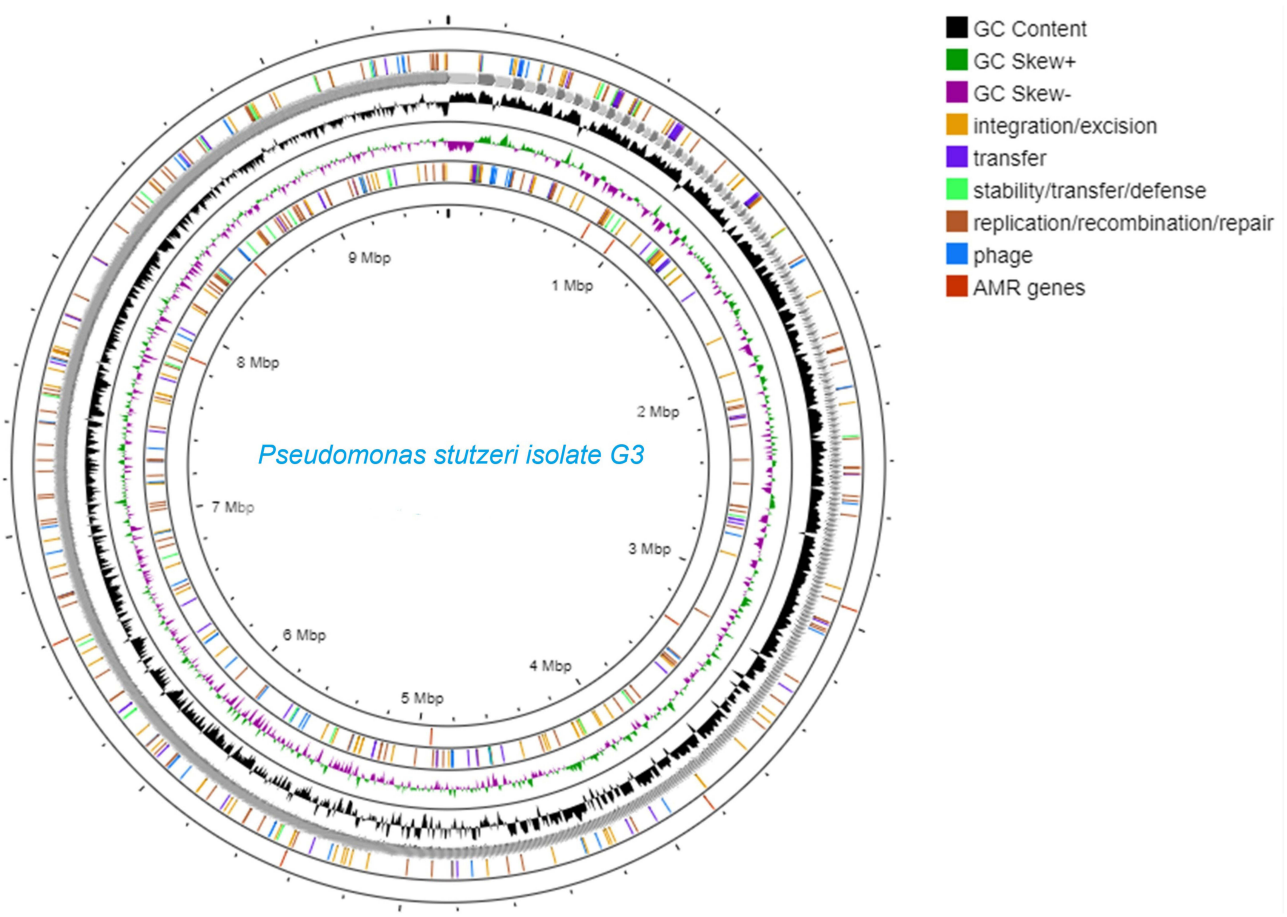

Figure 3 Graphical representation of the circular genome map of *Pseudomonas stutzeri* isolate G3 constructed in Proksee.[27] ARGs and mobile genetic elements were detected by the Comprehensive Antibiotic Resistance Database and mobileOG-db respectively.

Discussion

We isolated *Pseudomonas stutzeri* from Gunwade Lake, which exhibited resistance to several antibiotics, an unexpected finding for an environmental strain, given its presumably limited exposure to antibiotics.[2,35] To understand the molecular basis of multidrug resistance, we sequenced its genome and conducted in silico bioinformatic analyses to identify

Table 2 Antibiotic-Resistant Genes (ARGs) Identified in Pseudomonas Stutzeri Isolate G3 Using ResFinder and Comprehensive Antibiotic Resistance Databases (CARD)

Resistance Gene	% Identity	Resistance Phenotype	Contig	Position in Contig
PmpM	100	Fluoroquinolone antibiotic, Aminoglycoside antibiotic, Disinfecting Agents, Antiseptics	NODE_18	29,563–30051
adeF	61.11	Fluoroquinolone antibiotic, Tetracycline antibiotic	NODE_24	7150–10,326
			NODE_213	820–3957
			NODE_164	3529–6732
tmexC3	98.37	Tetracycline, Doxycycline, Minocycline, Tigecycline	NODE_84	1635–2798
tmexD2	99.14	Tetracycline, Doxycycline, Minocycline, Tigecycline	NODE_84	2814–5948

(*Continued*)

Table 2 (Continued).

Resistance Gene	% Identity	Resistance Phenotype	Contig	Position in Contig
toprJ3	99.93	Tetracycline, Doxycycline, Minocycline, Tigecycline	NODE_84	5953–7386
rsmA	98.36	Fluoroquinolone antibiotic, Diaminopyrimidine antibiotic, Phenicol antibiotic	NODE_284	1981–2166
OqxB	71.24	Ciprofloxacin, Nalidixic Acid, Trimethoprim, Chloramphenicol, benzalkonium chloride, cetylpyridinium chloride	NODE_309	4224–5480
vgb(A)	87.84	Quinupristin, Pristinamycin IA, Virginiamycin S	NODE_471	2380–3257
BcI	64.06	Cephalosporin, Penem	NODE_529	1816–2763
cipA	100	Lincosamide antibiotic, Streptogramin antibiotic, Streptogramin A antibiotic, Oxazolidinone antibiotic, Phenicol antibiotic, Pleuromutilin antibiotic	NODE_1207	1689–1814
OCH-2	100	Monobactam, Cephalosporin, Cephamycin, Penam, Penem	NODE_2091	1005–1139
tet(45)	84.87	Tetracycline, Doxycycline	NODE_3096	3–749

antibiotic-resistant genes. All resistance genes identified in isolate G3 were located on the chromosome, and the observed resistance phenotypes were aligned with the results of antibiotic susceptibility testing (Tables 1, 2, S1 and S2).

This suggests that antibiotic resistance could be intrinsic to *Pseudomonas stutzeri* which is why it thrives in diverse habitats, including hospitals.[2] *tmexC3D2-toprJ3*, which confers resistance to tetracycline, doxycycline, minocycline, and tigecycline, was also detected on the chromosome. *Pseudomonas stutzeri* T75, isolated from porcine faecal samples in Liaoning Province, China, harbours a plasmid containing the *tmexCD1-toprJ1* gene cluster on the plasmid pT75-VIM, rendering the strain resistant to tigecycline, an antibiotic of last resort.[6] Our study agrees with the suggestion that this gene cluster may have evolved from *Pseudomonas spp.*

Conversely, the *tmexC3D2-toprJ3* gene cluster found in isolate G3 may have originated from other sources, including agriculture, because tetracyclines are permitted for UK livestock farming. *P. stutzeri* can be a reservoir of multidrug resistance[1] by acquiring resistance gene cassettes from its environment through horizontal gene transfer of plasmids with integrons containing ARGs such as *In1998*[5] and incorporating them into their genome. In fact, tmexCD1-toprJ1 identified in *Pseudomonas stutzeri* T75 is flanked by two mobile elements, *ISPst3* and *ΔISPa86*[6] which could be involved in the translocation and transmission of *tmexCD1-toprJ1* to other species. The emergence of multidrug resistance in environmental

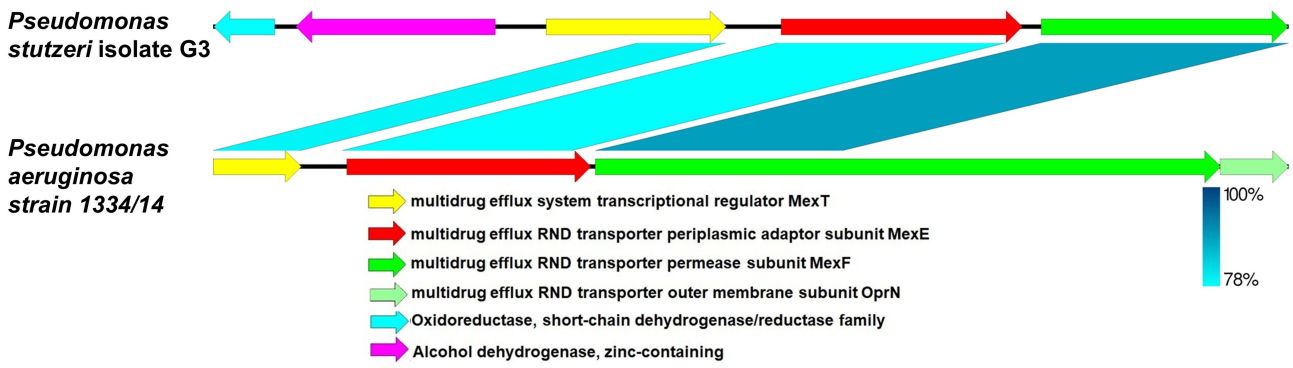

Figure 4 Comparison of the gene cluster in *Pseudomonas stutzeri* isolate G3 and *Pseudomonas aeruginosa* strain 1334/14 containing the Resistance-Nodulation-Division (RND) efflux pump, OqxB. Genes are indicated as arrows with different colours to note their functional classes. The length of each arrow represented a proportion of the gene size, and the direction of the arrow indicated the gene transcription direction. The shades between the two strains represent the sequence identity of linked regions Legend. *OqxB* gene location identified by Restfinder within the genomes of strains, isolate G3 as NODE_309, Position: 4224…5480, and on the chromosome of *Pseudomonas aeruginosa* strain 1334/14 (CP035739), Position: 1147921…1149305.

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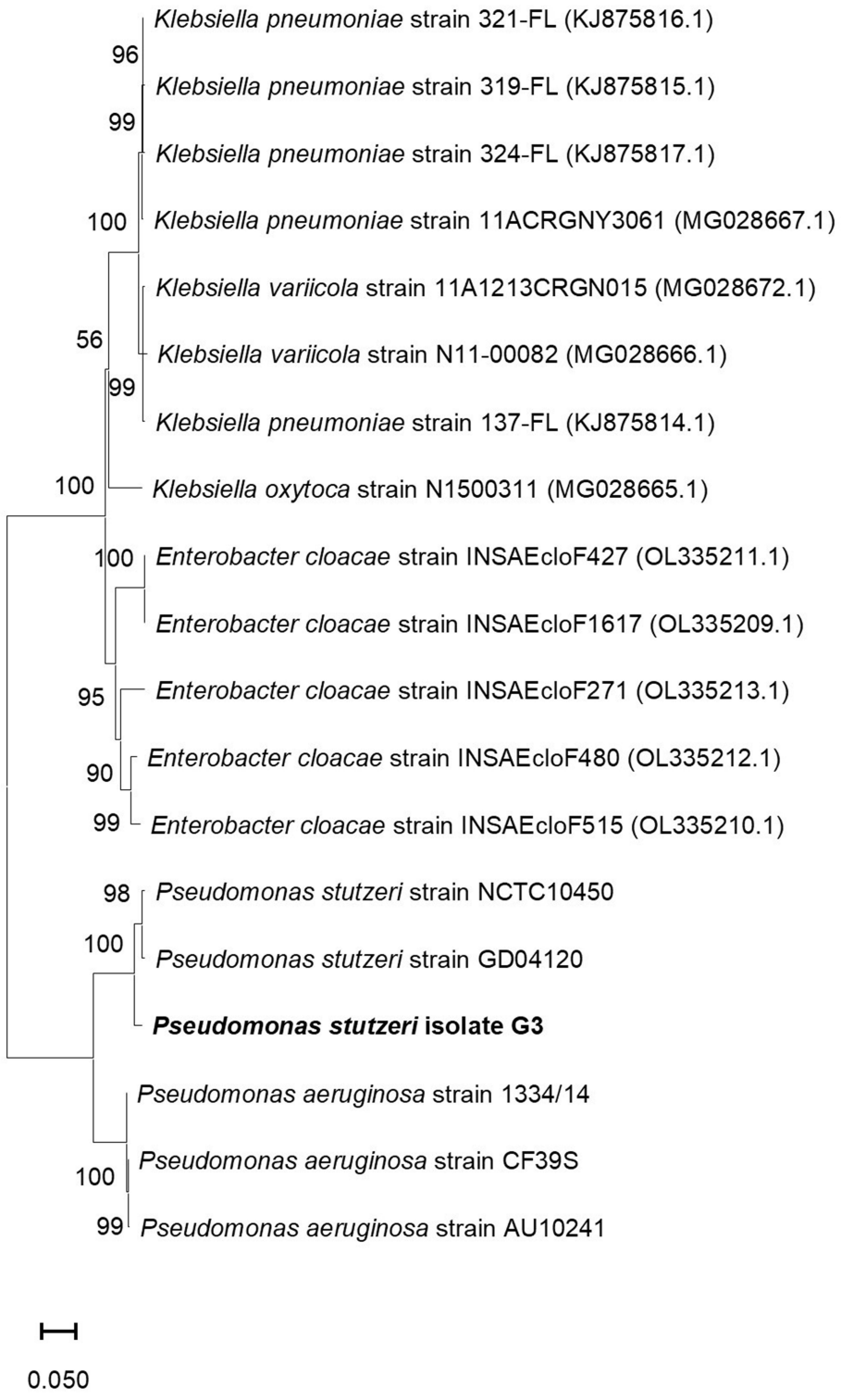

Figure 5 Phylogenetic relationship of *Pseudomonas stutzeri* isolate G3 and other species based on *Oqx*B gene analyses. The evolutionary history was inferred using the Neighbor-Joining method.[31] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.[32] The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method[33] and are in the units of the number of base substitutions per site. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1256 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.[25] The location of *OqxB* gene was identified by Restfinder within the genomes on the chromosomes of *Pseudomonas stutzeri* isolate G3 NODE_309, Position: 4224…5480; strain NCTC10450 (LR134319), Position 2360210…2361688; strain GD04120 (CP104580), Position 2768527…2769982; *Pseudomonas aeruginosa* strain 1334/14 (CP035739), Position: 1147921…1149305; strain CF39S (CP045916) Position 2962177…2963561; strain AU10241(CP130751) Position 2862548…2863932).

strains, such as *P. stutzeri* isolate G3, could pose a significant threat to public health because the populations of *P. stutzeri* identified in clinical settings usually come from the same population as the environmental isolates.[2]

Although the isolate was resistant to meropenem (Table 1), and beta-lactam antibiotics (Tables S1 and S2), we did not identify metallo-beta-lactamase (MBL) genes such as $bla_{\text{VIM-2}}$ in other *Pseudomonas stutzeri*.[1,5,6] However, we identified other genes in the draft genome of isolate G3, including *BcI, OCH-2*, and *oqxB* which could be involved in the multidrug resistance phenotype. *Oqx B* efflux pump is known to confer multidrug resistance in other Gram-negative organisms such as *K. pneumoniae, Escherichia coli, Enterobacter cloacae and Salmonella* spp.[8,12,13] We found genes encoding efflux pumps in other *Pseudomonas stutzeri* and *Pseudomonas aeruginosa* (Figure 4). The *oqxB* genes in *Pseudomonas* spp. were distinct from those in *Klebsiella* spp. and *Enterobacter* spp. (Figure 4). Our findings suggested that the *OqxB* efflux pump may be more widespread than previously thought.

We postulated that the efflux pump *oqxB* might be involved in multidrug resistance in *P. stutzeri* isolate G3 and other *Pseudomonas* spp. The *oqxB* sequence we found was not phylogenetically related to the TbtABM efflux pump previously reported in TBT-resistant *P. stutzeri* which made it resistant to nalidixic acid, chloramphenicol, and sulfamethoxazole.[7] The OqxB efflux pump had a higher efflux for the fluoroquinolone class of antibiotics (approximately 8-fold) than AcrB in *E.coli* C43 (DE3)ΔacrB.[12]

Furthermore, all oqxAB-positive *Enterobacteriaceae* strains were resistant to piperacillin, ceftriaxone, ceftizoxime, ciprofloxacin, and ceftazidime, while all strains were 100% susceptible to ciprofloxacin and norfloxacin.[11] While our findings provide a critical starting point, further research involving multiple isolates of *Pseudomonas* or database screening is necessary to confirm if the oqxB efflux pump plays a role in multidrug resistance in *Pseudomonas* spp as well. Notably, the absence of metallo-β-lactamase (MBL) genes, despite observed carbapenem resistance in *P. stutzeri*, suggests the involvement of alternative mechanisms, such as efflux pumps.

Our findings have important ecological, clinical, and public health implications. For instance, identifying the *tmexC3D2-toprJ3* tigecycline resistance cluster and other ARGs in *Pseudomonas stutzeri* significantly advances our understanding of environmental reservoirs of antibiotic resistance. The *tmexC3D2-toprJ3* cluster, which confers resistance to tigecycline, a last-resort antibiotic used to treat multidrug-resistant Gram-negative infections poses serious challenges in clinical settings. The co-occurrence of additional ARGs (eg, *PmpM, OCH-2, rsmA, oqxB*, and *CipA*), which confer resistance to fluoroquinolones, carbapenems, and aminoglycosides, complicates therapeutic strategies.

The spread of ARGs such as *tmexC3D2-toprJ3* emphasizes the urgent need for robust surveillance and control measures to curtail the dissemination of antimicrobial resistance. The detection of this resistance cluster in environmental bacterial strains underscores a possible pathway for resistance genes to transfer into human pathogens, representing a significant global health risk. Consequently, continuous monitoring, genomic investigations, and coordinated efforts are essential to better understand its epidemiology and design strategies to address its spread.

Conclusion

In summary, we report an MDR *Pseudomonas stutzeri* isolate G3 isolated from Gunwade Lake, located in Ferry Meadows, Peterborough's public-access nature park, which is the primary hub for water activities. Genomic analysis linked MDR profiles to ARGs such as *tmexC3D2-toprJ3* and *oqxB*. Although no MBL genes were detected, other resistance-associated genes (eg *BcI* and *OCH-2*) were identified, suggesting that alternative pathways may contribute to MDR phenotypes. This highlights *the* potential role of *P. stutzeri* as a reservoir of antibiotic resistance genes, facilitated by horizontal gene transfer.

These findings underscore the risk posed by environmental strains such as *P. stutzeri* isolate G3 in contributing to MDR in clinical settings, thus raising public health concerns. The broad dissemination of ARGs such as *oqxB* across species and its potential impact on resistance to fluoroquinolones and other antibiotics warrant further investigation to mitigate the threat posed by such MDR strains.

We suggest a multifaceted mitigating approach such as implementing stricter regulations on the use of antibiotics in agriculture and aquaculture to reduce environmental contamination. Additionally, enhancing wastewater treatment processes is crucial to effectively remove antibiotic residues and resistant microorganisms from effluents before they are released into the environment. Equally important are public awareness campaigns aimed at discouraging the improper

disposal of antibiotics and other pharmaceuticals, thereby minimizing their impact on the ecosystem. Together, these measures can help combat the growing threat of AMR and safeguard both public health and the environment.

While the identified ARGs provide insights into the multidrug resistance observed in the *P. stutzeri* G3 isolate during laboratory testing, functional gene expression studies are critical to establishing a causal relationship. Such efforts will help solidify our understanding and inform targeted interventions against antimicrobial resistance. Future directions include functional analyses to confirm gene expression and resistance phenotypes; investigating whether *oqxB* contributes to multidrug resistance in other *Pseudomonas* spp, as current knowledge is based on findings in other Gram-negative bacteria; conducting studies across multiple sites and isolates to account for seasonal or temporal variations in resistance patterns and extending sample collection timeframes to ensure comprehensive data on resistance dynamics.

Data Sharing Statement

The datasets used and analysed during this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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