



Draft Genome Sequence of Carbapenem-Resistant *Pseudomonas fluorescens* Strain BWKM6, Isolated from Feces of *Mareca penelope*

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ABSTRACT Migratory birds are potential vehicles of antibiotic-resistant bacteria. Here, we isolated the multidrug-resistant *Pseudomonas fluorescens* strain BWKM6 from the feces of *Mareca penelope*. The strain's draft genome sequence indicates that it harbors a metallo-beta-lactamase, a class C beta-lactamase, and several multidrug efflux pumps.

Many pathogenic human enteric bacteria have been isolated from wild birds (1). Studies have reported that antibiotic-resistant bacteria travel long distances via migratory birds (2). Owing to their ability to migrate long distances within short time periods, migratory birds are a potential source of antibiotic-resistant bacteria that may be transmitted to humans (3). One migratory bird, the Eurasian wigeon (*Mareca penelope*), breeds in the northernmost areas of Europe and Asia. The global population of *M. penelope* is estimated to be approximately 2.8 to 3.3 million (4). *M. penelope* leaves its breeding grounds during late summer to arrive in autumn at its wintering grounds across Europe and Asia. This species lives primarily in lakes and rivers and along coastlines, and it prefers a location near edible aquatic and terrestrial plants. The number of observed *M. penelope* individuals in Japan has been hundreds of thousands per year. However, the incidence and types of antibiotic-resistant bacteria associated with migratory birds in East Asia remain unknown.

The carbapenem-resistant *Pseudomonas fluorescens* strain BWKM6 was isolated on CHROMagar extended-spectrum beta-lactamase (ESBL) medium (Kanto Chemical Co., Inc., Tokyo, Japan) from the feces of *M. penelope*. The BWKM6 strain showed resistance to carbapenems, chloramphenicol, and tetracycline. *P. fluorescens* is found in a wide range of environments, including plants, soil, and water surfaces (5, 6). Different clinical strains of *P. fluorescens* have been reported as having high hemolytic activity, which induces cytotoxic and proinflammatory responses in epithelial intestinal cells (7, 8).

The draft genome sequence of *P. fluorescens* BWKM6 was analyzed by 100-bp paired-end sequencing on an Illumina HiSeq 2000 sequencing system (Hokkaido System Science Co., Ltd., Sapporo, Hokkaido, Japan). A total of 45,841,080 high-quality sequence reads was assembled *de novo* using CLC Genomics Workbench v6.5 (CLC Bio, Cambridge, MA). Approximately 99.2% of the sequenced reads were mapped again to the contigs. The final assembly of the genome produced 6,008,217 bp in 69 contigs, with an N_{50} value of 169,915 bp and a GC content of 60.1%. The assembled contigs were functionally annotated using the Rapid Annotations using Subsystems Technology (RAST) server (9). The genomes contained 5,335 putative coding sequences and 58 RNA genes.

The genome of BWKM6 encoded a metallo-beta-lactamase and a class C betalactamase. In addition, the genome encoded two multidrug-resistance proteins, streptothricin acetyltransferase and the fosfomycin resistance protein. It also encoded the Received 12 February 2018 Accepted 27 February 2018 Published 22 March 2018

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following multidrug-resistance efflux pumps: the resistance-nodulation-division efflux system membrane fusion protein/inner membrane transporter/outer membrane lipoprotein (CmeA, CmeB, and CmeC), the multidrug and toxic compound extrusion family of multidrug resistance efflux pumps, the MexC-MexD-OprJ multidrug efflux system, the MexE-MexF-OprN multidrug efflux system, the macrolide-specific efflux protein MacA, and the macrolide export ATP-binding/permease protein MacB.

These results suggest that *M. penelope* can spread multidrug-resistant *P. fluorescens* through migration between Japan and eastern Siberia and that the bacteria can be transmitted from birds to humans and vice versa. The genome of *P. fluorescens* BWKM6 will facilitate our understanding of the ecology and global spread of multidrug-resistant *P. fluorescens* via migratory birds (10–12).

Accession number(s). The draft genome sequence of *P. fluorescens* strain BWKM6 has been deposited in DDBJ/EMBL/GenBank under the accession number PPHS00000000.

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REFERENCES

- Hubálek Z. 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. J Wild Dis 40:639–659. https://doi.org/ 10.7589/0090-3558-40.4.639.
- Abulreesh HH, Goulder R, Scott GW. 2007. Wild birds and human pathogens in the context of ringing and migration. Ringing Migr 23:193–200. https://doi.org/10.1080/03078698.2007.9674363.
- Bonnedahl J, Järhult JD. 2014. Antibiotic resistance in wild birds. Ups J Med Sci 119:113–116. https://doi.org/10.3109/03009734.2014.905663.
- BirdLife International. 2017. Mareca penelope (amended version of 2016 assessment). The IUCN red list of threatened species 2017: eT22680157A111892532 IUCN, Gland, Switzerland. https://doi.org/10 .2305/IUCN.UK.2017-1.RLTS.T22680157A111892532.en.
- Bodilis J, Calbrix R, Guérillon J, Mérieau A, Pawlak B, Orange N, Barray S. 2004. Phylogenetic relationships between environmental and clinical isolates of *Pseudomonas fluorescens* and related species deduced from 16S rRNA gene and OprF protein sequences. Syst Appl Microbiol 27: 93–108. https://doi.org/10.1078/0723-2020-00253.
- Bossis E, Lemanceau P, Latour X, Gardan L. 2000. The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision. Agronomie 20:51–63. https://doi.org/10.1051/agro:2000112.
- Hsueh P, Teng LJ, Pan HJ, Chen YC, Sun CC, Ho SW, Luh KT. 1998. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. J Clin Microbiol 36:2914–2917.

- Sperandio D, Rossignol G, Guerillon J, Connil N, Orange N, Feuilloley MG, Merieau A. 2010. Cell-associated hemolysis activity in the clinical strain of *Pseudomonas fluorescens* MFN1032. BMC Microbiol 10:124. https://doi .org/10.1186/1471-2180-10-124.
- Aziz RK, Bartels D, Best AA, Dejongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
- Ma Q, Zhai Y, Schneider JC, Ramseier TM, Saier MH. 2003. Protein secretion systems of *Pseudomonas aeruginosa* and *P. fluorescens*. Biochim Biophys Acta 1611:223–233. https://doi.org/10.1016/S0005-2736(03)00059-2.
- Madi A, Lakhdari O, Blottière HM, Guyard-Nicodème M, Le Roux K, Groboillot A, Svinareff P, Doré J, Orange N, Feuilloley MG, Connil N. 2010. The clinical *Pseudomonas fluorescens* MFN1032 strain exerts a cytotoxic effect on epithelial intestinal cells and induces interleukin-8 via the AP-1 signaling pathway. BMC Microbiol 10:215. https://doi.org/10.1186/1471 -2180-10-215.
- Naghmouchi K, Le Lay C, Baah J, Drider D. 2012. Antibiotic and antimicrobial peptide combinations: synergistic inhibition of *Pseudomonas fluorescens* and antibiotic-resistant variants. Res Microbiol 163:101–108. https://doi.org/10.1016/j.resmic.2011.11.002.