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Detection of *bla*_{OXA-23}–Positive *Proteus mirabilis* Isolate, United States, 2024

Appendix

Supplementary Methods

AR Lab Network Testing

Public health laboratories (PHLs) in the Centers for Disease Control and Prevention Antimicrobial Resistance Laboratory Network perform routine testing including antimicrobial susceptibility testing (AST), phenotypic carbapenemase production testing, and real-time-PCR (RT-PCR) for detection of five targeted carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{IMP}, and *bla*_{VIM}), as previously described (1,2). Whole-genome sequencing (WGS) is performed to characterize a subset of isolates, including those with discordant carbapenemase production and RT-PCR results (i.e., carbapenemase production-positive but RT-PCR-negative for the five targeted carbapenemase genes), which may indicate the presence of novel carbapenemase genes. The Massachusetts PHL performed AST by Kirby-Bauer disk diffusion and phenotypic carbapenemase production testing by the modified carbapenemase inactivation method.

Genomic Analyses

Short-read WGS was performed at the Massachusetts PHL on an Illumina MiSeq platform using the Nextera DNA Library Prep Kit. Raw reads were processed as previously described (3) and the *bla*_{OXA-23} gene was identified using AMRFinderPlus (software v3.11.4; database v2023–02–23.1) (4). The CDC pipeline PHoeNIx v2.1.1 (<https://github.com/CDCgov/phoenix>) was used to process Illumina reads, perform genome assembly, and detect antimicrobial resistance genes (thresholds: 98% amino acid identity, 90%

coverage). Long-read WGS was performed at CDC using the Rapid Barcoding Kit V14 and an R10.4.1 flow cell on the MinION (Oxford Nanopore Technologies). Hybrid assembly was performed using Hybracter v0.11.0 (default settings hybrid-single with -auto) (5). BLASTn was used to identify the best match to the NCBI nucleotide database of the *bla*_{OXA-23}-containing region (6); alignment was generated using Easyfig (7). Phylogenetic tree was inferred based on alignment of the core genome performed using Parsnp v2.1.3 (default settings; reference strain: VAC [GCA_008041895.1]) (8). The tree was visualized using iTOL v7 (<https://itol.embl.de/>) (9) and rooted at the midpoint. WGS data were deposited to NCBI (BioSample: [SAMN41612253](#); Sample name: 2024DK-00154; MinION reads: [SRR33582377](#); Illumina reads: [SRR29245601](#); Hybrid assembly: [CP194042](#)).

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