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GENOME SEQUENCES

Complete Genome Sequences of Four Macrolide-Resistant Nondiphtheritic *Corynebacterium* Isolates

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ABSTRACT This report describes the complete genome sequences of four isolates of the nondiphtheritic *Corynebacterium* (NDC) species *Corynebacterium pseudodiphtheriticum* and *Corynebacterium propinquum*, recovered during investigation of a large diphtheria outbreak in Bangladesh. These data will assist in better delineating the boundary between these related species and understanding their virulence potential.

While toxigenic strains of *Corynebacterium diphtheriae* cause diphtheria, various nondiphtheritic *Corynebacterium* (NDC) species commonly colonize the skin and mucous membranes of various mammals (1). NDC are considered commensals but have gradually been recognized as opportunistic pathogens associated with endocarditis, pneumonitis, bronchiectasis, and skin infections (2–7). NDC identified as *Corynebacterium pseudodiphtheriticum* were codetected with *C. diphtheriae* in a large diphtheria outbreak reported previously (8). Subsequent whole-genome sequencing (WGS) revealed that 7.14% of the recovered NDC isolates were instead *Corynebacterium propinquum*, which is morphologically and biochemically similar (9). Isolates were selected to represent four unique biochemical profiles defined by API Coryne strips (bioMérieux, Durham, NC). Here, we report the complete genome sequences of three *C. pseudodiphtheriticum* isolates and one *C. propinquum* isolate to enrich the limited genomic resources of NDC species.

Isolates were grown from cryogenic stocks at CDC by streaking onto Trypticase soy agar with 5% sheep blood at 37°C for 24 h. Genomic DNA was extracted using the Maxwell RSC whole-blood DNA kit (Promega, San Luis Obispo, CA), further cleaned by salt/chloroform washing (10), and quantified using the Qubit double-stranded DNA (dsDNA) broad-range kit (Thermo Fisher Scientific, Waltham, MA). WGS was performed using both an Illumina MiSeq instrument (Illumina, San Diego, CA) and a PacBio Sequel II instrument (Pacific Biosciences, Menlo Park, CA). Illumina libraries were prepared using the NEBNext Ultra DNA library prep kit (New England Biolabs, Ipswich, MA), which resulted in DNA fragments of 500 to 1,000 bp for sequencing with the Illumina reagent kit v2 (500 cycles). PacBio libraries were prepared, following the selection of fragments of >20 kb with BluePippin (Sage Science, Beverly, MA, USA), using a SMRTbell template prep kit v2.0 and sequenced using the Sequel binding kit v2.0 with internal controls. The Illumina raw reads were checked for quality using FastQC v0.11.5 (11) and trimmed and filtered with Cutadapt v2.3 (-q 20,20 -m 50 –max-*n* = 2) (12). The PacBio reads were *de novo* assembled without filtering using Flye v2.9 (13), manually checked for circularity using Gepard v1.30 (14), and further polished by mapping them onto the Illumina trimmed reads using CLC Genomics Workbench v21 (CLC bio, Boston, MA, USA). The assembly completeness was

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TABLE 1 Characteristics of the four NDC isolates and genome assemblies in this study

Strain	Species	API code	Illumina		PacBio		Genome size (bp)	G+C content (%)	No. of CDSs	PC0752 = CD1121 for the GenBank accession no.	SRA accession no.
			No. of reads	Coverage (×)	No. of reads	Coverage (×)					
PC0752	*C. pseudodiphtheriticum*	5001004	1,552,280	152	65,122	418	2,374,277	55.4	2,078	CP091087	SRR17736613
PC1113	*C. propinquum*	7001004	1,218,404	117	108,487	644	2,514,362	56.6	2,185	CP091865	SRR17736611
PC1130	*C. pseudodiphtheriticum*	5101004	1,154,574	111	127,414	790	2,362,766	55.3	2,045	CP091864	SRR17736600
PC1145	*C. pseudodiphtheriticum*	7101004	2,658,698	258	104,700	630	2,402,479	55.2	2,094	CP091863	SRR17736585

evaluated using QUAST v5.0.2 (15). Finally, the assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (16). Default parameters were used for all software unless otherwise noted.

The genome of *C. propinquum* PC1113 was distinguishable in length, G+C content, and number of predicted protein-coding sequences (CDSs) (Table 1). The average nucleotide identity (ANI) between PC1113 and *C. propinquum* reference genomes (GenBank accession numbers CP068160 and CP068161) was 97.6%, while the ANI between PC1113 and the three *C. pseudodiphtheriticum* genomes here averaged 86.8%, consistent with their species assignment based on a 95% threshold (17). All four genomes encoded *ermX*, a determinant of macrolide resistance (18). Compared with the virulence factor profile of *C. diphtheriae* NCTC13129 (NC_002935.2), all four encoded similar iron uptake systems but lacked the adherence pili. A further query against the Virulence Factor Database (VFDB) (19) predicted genes encoding acid resistance, antiphagocytosis, and copper uptake, leaving much to learn about the ecology of NDC species.

Data availability. The trimmed sequencing reads have been deposited at the NCBI Sequence Read Archive under accession numbers SRR17736613, SRR17736611, SRR17736600, and SRR17736585. The complete genome sequences have been deposited at GenBank under the accession numbers CP091087.1, CP091865.1, CP091864.1, and CP091863.1. The versions described in this paper are the first versions.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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