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CULTURE COLLECTIONS/
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A Culture Collection of 50 *Neisseria gonorrhoeae* Isolates

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ABSTRACT A culture collection of 50 *Neisseria gonorrhoeae* isolates is available from the CDC & FDA Antibiotic Resistance Isolate Bank. Associated data include antibiotic susceptibility information for azithromycin, cefixime, cefpodoxime, ceftriaxone, tetracycline, ciprofloxacin, penicillin, and spectinomycin and linked whole-genome sequences.

Antibiotic resistance is a major concern for clinical management of gonorrhea. The ability of *Neisseria gonorrhoeae* to shuffle and exchange its genes and to acquire antibiotic resistance is of particular global concern (1–3). At present in the United States, ceftriaxone in combination with azithromycin is the only first-line choice for treating uncomplicated *N. gonorrhoeae* infections (2, 4). Combating the spread of antibiotic resistance in *N. gonorrhoeae* requires an array of tools that include but are not limited to effective diagnostic tests and methods to improve treatment by detecting emerging resistance traits.

As part of the solution to combat antibiotic resistance, the CDC is providing a collection of *N. gonorrhoeae* strains (5–7). This panel consists of 50 isolates selected from the 2012 Gonococcal Isolate Surveillance Project (GISP) sentinel sites (8). *N. gonorrhoeae* strains in this panel have diverse antibiotic susceptibility profiles and were collected from various regions across the United States (Table 1). The antibiotics included in the profile are azithromycin, ceftriaxone, cefixime, ciprofloxacin, cefpodoxime, tetracycline, penicillin, and spectinomycin (7). Antimicrobial susceptibility testing (AST) was performed according to the Clinical and Laboratory Standards Institute (CLSI) M07 protocol (9, 10) and following Clinical Laboratory Improvement Amendments (CLIA) regulations.

To facilitate the development of genetic testing, the complete genome sequencing data for each isolate are also provided. DNA was extracted using the Promega genomic DNA purification kit (Promega, Madison, WI), and whole-genome sequencing was performed using a standard protocol; default parameters were used throughout (11). Specifically, libraries were prepared using the NEB genome library preparation kit (New England Biolabs, MA) and sequenced as 2 × 150-bp paired-end reads using the HiSeq 2500 platform (Illumina, CA). Raw sequence reads were analyzed in three stages. Preprocessing assessed the read quality with Trim Galore (v0.3.7), which contains FastQC and Cutadapt (12) to perform quality assessment, removal of duplicate reads, and trimming of reads. The genomes were assembled using SPAdes (v3.12.0) (13) and QUAST (v4.3) (14). Finally, annotation was completed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (15, 16). Reads were mapped to the FA19 reference sequence (GenBank accession number CP012026). This *N. gonorrhoeae* isolate panel and associated AST data and genomic information are available on the website of the CDC & FDA Antibiotic Resistance Isolate Bank (https://wwwn.cdc.gov/ARIsolateBank/Panel/PanelDetail?ID=11) (Table 1) (7).

The *N. gonorrhoeae* panel is provided as frozen isolates preserved in 300 to 500 μl of Trypticase soy broth (TSB) medium containing 20% glycerol and maintained at −70°C. To revive the frozen cultures, we recommend using a sterile cotton applicator

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TABLE 1 Isolates in the culture collection, with Antibiotic Resistance Isolate Bank accession numbers, AST data, characteristics of genome raw data, and assembly accession numbers

Antibiotic Resistance Isolate Bank no.	HHS region[a]	AST MIC (μg/ml) for[b]:								SRA accession no. (raw reads)	Genome assembly accession no.	No. of reads	N_{50} (bp)	GC content (%)	No. of contigs
		AZI	CFX	CPD	CRO	TET	CIP	PEN	SPE						
165	4	1	0.25	2	0.06	8	8	4	S	ERR854938	SAVC00000000	4,834,238	46,424	52.41	107
166	4	1	0.25	2	0.125	4	8	4	S	ERR854921	SAVD00000000	5,193,304	43,861	52.4	110
167	4	8	0.03	0.03	0.008	1	0.015	0.25	S	ERR855352	SAVE00000000	5,660,874	44,069	52.63	110
168	4	0.5	0.25	1	0.06	4	16	4	S	ERR854924	SAVF00000000	5,971,046	41,042	52.4	113
169	5	1	0.25	1	0.06	4	16	2	S	ERR854922	SAVG00000000	5,341,880	40,976	52.4	113
170	5	1	0.25	1	0.06	4	16	2	S	ERR854897	SAVH00000000	4,784,296	40,704	52.4	114
171	5	0.5	0.25	1	0.06	4	16	2	S	ERR854937	SAVI00000000	4,902,936	41,042	52.41	113
172	5	0.5	0.25	1	0.06	2	16	2	S	ERR854906	SAVJ00000000	5,135,014	46,566	52.39	112
173	5	0.5	0.25	2	0.125	4	16	2	S	ERR854907	SAVK00000000	4,886,630	40,767	52.42	110
174	5	1	0.25	2	0.125	4	16	4	S	ERR854900	SAVL00000000	4,649,238	46,424	52.41	114
175	5	16	0.015	0.02	0.008	1	0.015	0.25	S	ERR855365	SAVM00000000	5,010,650	44,146	52.62	109
176	8	0.5	0.25	1	0.03	4	16	4	S	ERR854869	SAVN00000000	4,617,488	46,744	52.4	108
177	6	2	0.03	0.06	0.015	2	0.03	1	S	ERR855355	SAVO00000000	5,564,656	47,536	52.63	107
178	6	1	0.25	2	0.06	8	16	4	S	ERR855325	SAVP00000000	4,746,854	44,276	52.63	117
179	6	8	0.015	0.02	0.008	1	0.015	0.25	S	ERR855356	SAVQ00000000	5,737,628	41,328	52.62	106
180	8	0.5	0.25	1	0.06	4	16	2	S	ERR854913	SAVR00000000	5,267,970	44,124	52.41	112
181	9	256	0.06	0.13	0.03	2	0.015	0.5	S	ERR855357	SAVS00000000	5,417,388	44,564	52.62	106
182	9	0.5	0.25	1	0.06	4	16	2	S	ERR854870	SAVT00000000	4,644,886	44,221	52.4	107
183	9	1	0.25	1	0.06	4	16	2	S	ERR854902	SAWW00000000	5,209,558	44,200	52.42	113
184	4	0.5	0.25	1	0.06	4	16	2	S	ERR854916	SAVU00000000	4,992,592	47,291	52.39	106
185	4	0.5	0.25	1	0.06	4	16	2	S	ERR854898	SAVV00000000	5,294,702	46,419	52.42	114
186	9	0.5	0.25	2	0.125	4	16	2	S	ERR854919	SAVW00000000	5,494,734	44,219	52.39	122
187	9	2	0.125	0.5	0.03	1	4	2	S	ERR855360	SAVX00000000	5,563,168	49,484	52.4	108
188	9	1	0.25	1	0.06	4	16	2	S	ERR854932	SAVY00000000	5,511,962	44,123	52.4	108
189	4	0.5	0.25	2	0.06	4	8	4	S	ERR854903	SAVZ00000000	4,952,236	44,172	52.41	110
190	9	1	0.25	2	0.125	4	16	2	S	ERR854936	SAWX00000000	5,463,010	43,459	52.4	119
191	6	1	0.25	2	0.06	4	16	8	S	ERR854899	SAWA00000000	5,102,370	41,042	52.41	118
192	2	1	0.25	2	0.06	8	8	4	S	ERR854917	SAWB00000000	5,246,534	46,428	52.4	114
193	2	2	0.03	0.13	0.03	2	0.015	2	S	ERR855351	SAWC00000000	5,333,106	47,350	52.51	98
194	7	0.5	1	2	0.5	1	0.015	2	S	ERR854905	SAWD00000000	6,314,448	52,028	52.44	103
195	9	1	0.25	1	0.06	4	16	4	S	ERR854928	SAWE00000000	5,389,894	40,207	52.34	170
196	3	0.5	0.25	1	0.03	2	8	4	S	ERR854927	SAWF00000000	5,276,258	46,791	52.46	102
197	3	4	0.125	0.5	0.03	1	16	2	S	ERR855353	SAWG00000000	5,571,124	46,772	52.39	116
198	5	1	0.25	1	0.06	4	16	2	S	ERR854904	SAWH00000000	4,901,472	46,791	52.46	106
199	5	2	0.03	0.06	0.03	2	0.015	2	S	ERR855358	SAWI00000000	4,732,042	47,812	52.51	101
200	10	0.5	0.25	2	0.125	2	16	4	S	ERR854929	SAWJ00000000	5,321,716	47,280	52.4	116
201	10	0.5	0.25	1	0.125	2	8	2	S	ERR854912	SAWK00000000	4,494,320	47,257	52.41	108
202	10	16	0.015	0.02	0.008	1	0.015	0.25	S	ERR855359	SAWL00000000	4,398,840	47,380	52.64	108
203	10	0.5	0.25	2	0.125	4	16	2	S	ERR854908	SAWM00000000	6,022,354	41,053	52.4	114
204	10	0.5	0.25	1	0.06	4	16	2	S	ERR854867	RQJE00000000	5,355,056	46,767	52.4	104
205	9	0.5	0.25	1	0.06	2	16	2	S	ERR956689	SSHK00000000	4,447,980	43,642	52.01	178
206	9	0.5	0.25	1	0.06	4	16	2	S	ERR956690	SAWN00000000	5,067,848	46,426	52.4	118
207	9	0.5	0.25	1	0.06	4	16	2	S	ERR855324	SAWO00000000	5,906,290	46,565	52.4	102
208	9	1	0.25	0.5	0.06	4	16	2	S	ERR854930	SAWP00000000	4,949,220	47,280	52.46	107
209	9	1	0.25	2	0.06	2	16	4	S	ERR854933	SAWQ00000000	4,802,186	46,795	52.41	110
210	9	0.3	0.25	2	0.06	1	16	2	S	ERR854911	SAWR00000000	4,983,750	46,772	52.4	116
211	9	1	0.25	1	0.06	2	16	4	S	ERR854910	SAWS00000000	5,147,574	47,501	52.4	113
212	10	0.5	0.25	1	0.06	4	16	2	S	ERR854939	SAWT00000000	5,684,566	46,909	52.41	110
213	10	0.5	0.25	1	0.06	4	16	2	S	ERR854920	SAWU00000000	5,034,160	46,770	52.4	108
214	10	0.5	0.25	2	0.125	4	16	2	S	ERR854935	SAWV00000000	5,608,468	44,114	52.41	107

[a] Department of Health and Human Services (HHS) region (https://www.hhs.gov/about/agencies/iea/regional-offices/index.html).
[b] AZI, azithromycin; CFX, cefixime; CPD, cefpodoxime; CRO, ceftriaxone; TET, tetracycline; CIP, ciprofloxacin; PEN, penicillin; SPE, spectinomycin; S, susceptible.

or an inoculating loop to transfer a small amount (e.g., 50-μl equivalent) of the frozen culture onto an *N. gonorrhoeae* medium base supplemented with 1% IsoVitaleX or a chocolate II agar plate (BBL/Becton, Dickinson) following standard microbiological procedures. The plates should be incubated at 36°C ± 1°C for 20 to 24 h in a humidified chamber supplemented with 5% CO_2.

Data availability. The GenBank and SRA accession numbers for the isolates are provided in Table 1. To acquire the panel, or for questions or suggestions, please

contact the CDC & FDA Antibiotic Resistance Isolate Bank directly (https://wwwn.cdc.gov/ARIsolateBank) (7).

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