Draft Genome Sequences of *Vibrio cholerae* Non-O1, Non-O139 Isolates from Common Tern Chicks (*Sterna hirundo*) following a Mass Mortality Event

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**ABSTRACT** *Vibrio cholerae* is an inhabitant of aquatic environments worldwide. Here, we report the draft genome sequences of eight *V. cholera* non-O1, non-O139 isolates that were recovered from the corpses of two seabird chicks (common terns) following a mass mortality event in a German breeding colony in 2019.

The presence of *Vibrio cholerae* in seabirds has been well known for many years (1, 2). Migrating birds are regarded as vectors for long-distance transport of these bacteria (3). Usually, the bacteria are regarded as commensals, while diseases of birds caused by *V. cholerae* are rarely reported.

In July 2019, a high chick mortality rate was observed in a colony of common terns (*Sterna hirundo*) in the saltmarshes of Neufelderkoog (District Dithmarschen) in the River Elbe Estuary (53°53’37.0"N, 8°58’55.21"E) (4). There, the seabird brood of 1 year (~1,500 chicks) died within 1 week shortly before they were able to fly. To determine the cause of death, necropsies and microbiological investigations were conducted on two chick corpses. Tissue samples were taken from inner organs, and the samples were cultivated at 37°C on Columbia sheep blood agar (Thermo Fisher Scientific, Berlin, Germany) and MacConkey agar (Merck, Darmstadt, Germany) for 24 h and 48 h, respectively. Visible colonies were investigated using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper; Bruker Daltonik, Bremen, Germany). *V. cholerae* non-O1, non-O139 isolates were recovered from liver, kidney, heart, small intestine, and lung, raising the possibility that these bacteria could be involved in the death of the birds. Herring as the sole feed for the chicks were suspected as a source of the infection. One *V. cholerae* isolate from each organ (eight isolates in total) (Table 1) was cultured on thiosulfate-citrate-bile-sucrose agar (Thermo Fisher Scientific) and ChromID *Vibrio* agar (bioMérieux, Marcy-l’Etoile, France) and sent to the Federal Institute for Risk Assessment.

For whole-genome sequencing, isolates were grown in lysogeny broth and genomic DNA was extracted with the PureLink genomic DNA kit (Invitrogen, Karlsruhe, Germany). MiSeq whole-genome sequencing (5) was conducted using the Nextera XT DNA sample preparation kit for library preparation and the MiSeq reagent 600-cycle v3 kit for paired-end sequence determination, as specified by the manufacturer (Illumina, Inc., San Diego, CA, USA). Raw reads were processed to quality-trimmed sequences using fastp v0.19.5 (https://github.com/OpenGene/fastp) with the following specifications: base limit, 50; length required, 15. Sequences were further checked with FastQC v1.0.4 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Automated de novo assembly (SPAdes v3.5.49) and genome annotation were performed using PATRIC (release 3.5.39) (6). Default parameters were routinely used for all

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| Parameter                      | Data for isolate: |
|-------------------------------|-------------------|
| Isolation origin             | V917-19 | V918-19 | V919-19 | V920-19 | V921-19 | V922-19 | V923-19 | V924-19 |
| Country of isolation          | Germany | Germany | Germany | Germany | Germany | Germany | Germany | Germany |
| Yr of isolation              | 2019    | 2019    | 2019    | 2019    | 2019    | 2019    | 2019    | 2019    |
| Phenotypic resistance         | None    | None    | None    | None    | None    | None    | None    | None    |
| Ph (mg/liter)                 | None    | None    | None    | None    | None    | None    | None    | None    |
| Ampicillin                    | 8       | 4       | 4       | 4       | 4       | 4       | 4       | 4       |
| Azithromycin                  | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      |
| Cefepime                      | 0.25    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    |
| Chloramphenicol               | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      |
| Ciprofloxacin                 | ≤0.015  | ≤0.015  | ≤0.015  | ≤0.015  | ≤0.015  | ≤0.015  | ≤0.015  | ≤0.015  |
| Colistin                      | >16     | >16     | >16     | >16     | >16     | >16     | >16     | >16     |
| Ertapenem                     | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    | 0.12    |
| Cefotaxime                    | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   |
| Cefoxitin                     | 4       | 4       | 4       | 8       | 8       | 8       | 8       | 8       |
| Gentamicin                    | 2       | 1       | 1       | 2       | 1       | 2       | 2       | 1       |
| Imipenem                      | 2       | 2       | 2       | 2       | 2       | 2       | 2       | 2       |
| Meropenem                     | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    | 0.25    |
| Nalidixic acid                | ≤4      | ≤4      | ≤4      | ≤4      | ≤4      | ≤4      | ≤4      | ≤4      |
| Sulfamethoxazole              | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      | ≤8      |
| Cefotaxime-clavulanic acid    | ≤0.06   | ≤0.06   | ≤0.06   | ≤0.06   | ≤0.06   | ≤0.06   | ≤0.06   | ≤0.06   |
| Ceftazidime                   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   |
| Ceftazidine-clavulanic acid   | 0.25    | ≤0.12   | ≤0.12   | ≤0.12   | ≤0.12   | ≤0.12   | ≤0.12   | ≤0.12   |
| Temocillin                    | 4       | 2       | 2       | 2       | 2       | 2       | 2       | 2       |
| Tetracycline                  | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      | ≤2      |
| Tigecycline                   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   |
| Trimethoprim                  | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   | ≤0.25   |
| Sequencing parameters         |          |          |          |          |          |          |          |          |
| No. of reads (total)          | 1,483,180 | 1,727,876 | 2,045,808 | 1,406,760 | 1,002,364 | 1,206,780 | 1,483,552 | 1,206,780 |
| Average read length (bp)      | 270     | 274     | 275     | 275     | 276     | 273     | 274     | 273     |
| No. of contigs                | 55      | 56      | 59      | 56      | 60      | 64      | 71      | 61      |
| $N_{50}$ (bp)                 | 318,246 | 688,697 | 688,696 | 318,246 | 318,246 | 318,246 | 318,246 | 324,219 |
| $L_{50}$                      | 4       | 3       | 3       | 4       | 4       | 4       | 4       | 4       |
| Genome coverage (×)           | 25      | 28      | 30      | 25      | 20      | 20      | 23      | 20      |
| SRA accession no.             | SRR12520475 | SRR12520474 | SRR12520473 | SRR12520472 | SRR12520471 | SRR12520470 | SRR12520469 | SRR12520468 |
| Genomic features              |          |          |          |          |          |          |          |          |
| Genome size (bp)              | 4,089,403 | 4,076,756 | 4,089,668 | 4,088,449 | 4,089,048 | 4,088,105 | 4,089,034 | 4,089,730 |
| GC content (%)                | 47.43   | 47.44   | 47.44   | 47.43   | 47.43   | 47.43   | 47.43   | 47.44   |
| Total no. of genes            | 3,985   | 3,971   | 3,987   | 3,984   | 3,990   | 3,992   | 3,988   | 3,990   |
| No. of coding genes           | 3,779   | 3,764   | 3,781   | 3,780   | 3,782   | 3,786   | 3,780   | 3,783   |
| No. of CDSs (total)           | 3,872   | 3,857   | 3,874   | 3,872   | 3,877   | 3,880   | 3,874   | 3,876   |

(Continued on next page)
### TABLE 1 (Continued)

| Parameter                          | Data for isolate: | V917-19 | V918-19 | V919-19 | V920-19 | V921-19 | V922-19 | V923-19 | V924-19 |
|------------------------------------|--------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| No. of CDSs (coding)               |                    | 3,779   | 3,764   | 3,781   | 3,780   | 3,782   | 3,786   | 3,780   | 3,783   |
| Total no. of RNA genes             |                    | 113     | 114     | 113     | 112     | 113     | 112     | 114     | 114     |
| Total no. of rRNA genes (5S, 16S, 23S) |                  | 7, 7, 3 | 7, 7, 4 | 7, 7, 3 | 7, 7, 4 | 7, 7, 4 | 7, 7, 4 | 7, 7, 4 | 7, 7, 4 |
| No. of complete rRNA genes         |                    | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 | 7, 1, 1 |
| No. of partial rRNA genes          |                    | 0, 6, 2 | 0, 6, 3 | 0, 6, 2 | 0, 6, 3 | 0, 6, 3 | 0, 6, 3 | 0, 6, 3 | 0, 6, 3 |
| No. of tRNA genes                  |                    | 92      | 92      | 92      | 91      | 91      | 91      | 92      | 92      |
| Total no. of pseudogenes           |                    | 93      | 93      | 93      | 92      | 95      | 94      | 94      | 93      |
| No. of predicted prophages          |                    | 2       | 1       | 2       | 2       | 2       | 2       | 2       | 2       |
| 47.7-kb K139 (GenBank accession no. NC_003313) |          | +       | +       | +       | +       | +       | +       | +       | +       |
| 7.1-kb KSF-1 phi (GenBank accession no. AY714348.1) |          | +       | –       | +       | +       | +       | +       | +       | +       |
| Plasmidsa                          |                    | ND      | ND      | ND      | ND      | ND      | ND      | ND      | ND      |
| Acquired antimicrobial resistanceb |                    | None    | None    | None    | None    | None    | None    | None    | None    |
| Sequence typec                     |                    | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown |
| BioProject no.                     |                    | PRINAS563188 | PRINAS563189 | PRINAS563190 | PRINAS563191 | PRINAS563192 | PRINAS563193 | PRINAS563194 | PRINAS563195 |
| BioSample no.                      |                    | SAMN12670120 | SAMN12670121 | SAMN12670122 | SAMN12670123 | SAMN12670124 | SAMN12670125 | SAMN12670126 | SAMN12670133 |
| GenBank accession no.              |                    | VTWK00000000.1 | VTWL00000000 | VTWM00000000.1 | VTWN00000000.1 | VTWO00000000.1 | VTWP00000000.1 | VTWQ00000000.1 | VTWR00000000.1 |

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*a* MICs were determined using broth microdilution according to the Clinical and Laboratory Standards Institute guidelines (13).

*b* CDSs, coding sequences.

*c* Analysis was conducted using PHASTER (https://phaster.ca) with default parameters. +, present; –, absent.

*d* Analysis was conducted using PlasmidFinder v2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder) with a 95% threshold for minimum identity and 60% minimum coverage. ND, not detected.

*e* Analysis was conducted using ResFinder v3.0 (https://cge.cbs.dtu.dk/services/ResFinder) with a 90% threshold for identity and 60% minimum length. The analysis of acquired determinants for the antimicrobial classes of aminoglycosides, β-lactams, colistin, fosfomycin, fusidic acid, macrolides, nitroimidazoles, oxazolidinones, phenicols, rifampin, sulfonamides, tetracyclines, trimethoprim, and glycopeptides yielded no matches.

*f* Analysis was conducted using MLST v2.0 (https://cge.cbs.dtu.dk/services/MLST) using the *Vibrio cholerae* scheme. All strains had identical alleles, as follows: adk114, 100% identity; gyrB30, 100% identity; mdhE97, 100% identity; metE123, 100% identity; pntA66, 100% identity; purM9, 100% identity; pyrC (novel allele), 99.78% identity to pyrC147.
software tools. Further information on software versions and parameters is given in Table 1. Bioinformatic analysis was conducted with the specified tools of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org) and PGAP (National Center for Biotechnology Information) (7). Prophage prediction was performed with PHASTER (accessed 9 June 2019) (8).

Important phenotypic and genotypic features of the V. cholerae genomes are summarized in Table 1. Determination of antimicrobial resistance phenotypes was performed as described previously (9). Because the genomes exhibited <19 single-nucleotide polymorphisms (SNPs) in 4,072,405 positions (representing nearly 100% of the genomes), the isolates were suggested to be clonal.

In silico prediction of phage-associated sequences revealed the presence of up to two prophages. One prophage sequence is similar to that of the linear V. cholerae phage KSF-1 phi (GenBank accession number AY714348) (10). The second prophage possesses sequences matching those of the phage myovirus K139 (GenBank accession number NC_003313) (11).

The genomes of the seabird isolates possess an SXT/R391-like integrative conjugative element (ICE) that is related to a 103-kb ICEVchBan8 element (GenBank accession number JQ345361) of a human pathogenic V. cholerae O37 strain (12). This ICE encodes potential virulence factors in a hot spot region of 45 kb, which might have contributed to the premature deaths of the young birds.

Data availability. Accession numbers for whole-genome sequences and raw sequencing reads (SRA accession numbers) are listed in Table 1.

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