Candida vulturna Outbreak Caused by Cluster of Multidrug-Resistant Strains, China

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Appendix

Additional Case Details

Case 4 was initially admitted to the department of geriatrics; Case 6 the department of general surgery; Cases 7 and 10 the department of neurosurgery; and Case 8 the department of orthopedics. These patients were later transferred to the ICU of the hospital. The earliest two C. vulturna infection cases were identified in the neuroscience ward, we suspect that C. vulturna was transmitted from other wards to the ICU.

Some patients were initially admitted to the general surgery, neuroscience, or other wards of the hospital prior to transfer to the ICU. Since the earliest two C. vulturna infection cases were identified in the neuroscience ward, we suspect that C. vulturna was transmitted from other wards to the ICU.

There could be multiple reasons for the reduction of infection cases during COVID. First, during COVID, the disinfectant with an increased concentration of hypochlorite (two-fold) was
used for floor disinfection. Second, disinfectants (such as 75% alcohol in the form of sprays and wipes) were available for all visitors and healthcare staff throughout the hospital. Third, the general and ICU wards strictly limited visitors.

**Materials and methods**

**Strains and culture conditions**

*C. vulturna*, *C. auris*, and *C. haemuloni* strains were routinely grown on solid YPD medium (2% Glucose, 2% peptone, 1% yeast extract, 2% agar). Modified Lee’s glucose media (1) was used for *Candida* aggregation and biofilm assays.

For growth on nutrient agar, approximately 150 cells were plated on Lee’s glucose medium and cultured at 30°C or 37°C for 3 days. For liquid culture, fungal cells were inoculated into 3 mL Lee’s glucose liquid medium to an OD$_{600}$ of 0.2 and incubated at 30°C or 37°C with shaking for 24 hours.

Environmental screening assays were performed to isolate *C. vulturna* from hospital surfaces, including walls, floors, bedside tables, bed sheets, bed rails, bed frames, blood-pressure cuffs, and chairs. More than 300 environmental samples were analyzed. Swab and wipe samples were used for culture assays on CHROMagar *Candida* medium.

To develop biofilms on silicone squares, approximately 2 x 10$^6$ cells of each strain were inoculated into each well containing one silicone square (10 mm x 10 mm, Bentec Medical, INC., Woodland) and 600 μL Lee’s glucose medium. After incubation for 48 hours at 30°C with shaking, the silicone squares were washed gently with ddH$_2$O three times and used for scanning electron microscopy (SEM) assays.

**SEM assays**

SEM assays were performed as described in our previous publication (2). Briefly, the silicone squares with *Candida* biofilms were fixed with 2.5% glutaraldehyde. The samples were
dehydrated in increasing concentrations of ethanol (25%-50%-70%-90%-100%), followed by tert-butyl alcohol solvent displacement through a series of increasing concentration of tert-butyl alcohol (25%-50%-70%-90%-100%) and freeze-dried. Finally, the samples were coated with gold and imaged using a scanning electron microscope (FlexSEM 1000 II, HITACHI).

**Antifungal drug susceptibility assays**

Minimum inhibitory concentrations (MICs) were determined according to the CLSI (Clinical Laboratory Standards Institute, 2012) guidelines. Liquid RPMI-1640 medium (w/v, 1.04% RPMI-1640, 3.45% MOPs, pH was adjusted to 7.0) containing a series of concentrations of different antifungal drugs was used. MICs were determined after 24 hours incubation at 35°C. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 served as quality controls.

**ITS- and MLST-based species identification and phylogenetic analysis**

*C. vulturna* strains were streaked and grown on YPD plates. Genomic DNA of single colonies was extracted for PCR analysis. A fragment containing the internal transcribed spacer (ITS), partial 18S small subunit (SSU), and 28S large subunit (LSU) ribosomal sequences were amplified using the primer pair ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) / NL4 (5’-GGTCCGTGTTTCAAGACGG-3’). Eight genes (*AAT1, ACC1, ADP1, ALA1, ERG11, RPB1, RPB2, and ZWF1*) were chosen for MLST analysis based on prior studies (3, 4). The following primers were used in the PCR reactions:

**AAT1** (540 bp):

AAT1F: aaggagtacaggggtagc, AAT1R: aacgagctgtctaatccttc

**ACC1** (515 bp):

CvACC1F: accaacaacacaactcge, CvACC1R: ccaacactagtcatgaa

**ADP1** (499 bp):

ADP1F: ttcacaagccacacag, ADP1R: acacttcaccggaatgt
ALA1 (530 bp):

ALA1F: tgcgaactccaaagtgta, ALA1R: ttcaaaaccataccgggtg

ERG11 (562 bp):

ERG11F: aactctcgtttgatggagca, ERG11R: aatgcaacaagaaccaagca

RPB1 (516 bp):

RPB1F: agaagagatattaatgcggtg, RPB1R: ccatgtatgtagcaacgtga

RPB2 (513 bp):

RPB2F: atcgaggagaaggtggagaa, RPB2R: ttcctcaacacaggcttca

ZWF1 (503 bp):

ZWF1F: actcgtctatcctgaaggtg, CvZWF1R: tctcgctgcaaggttagat

The PCR products were sequenced and analyzed. The homologous sequences of representative species of the CTG clade were retrieved from the NCBI GeneBank or CGD (http://www.candidagenome.org/) databases. The sequences of C. vulturna isolates and other CTG species were analyzed using software mafft v7.015b (5). The phylogenetic tree was generated using the programme RAxML v7.3.2 (6). The General Time Reversible (GTR) model, gamma distribution, and 1000 bootstraps were adopted.

**Sequence information for strains CVDH01-CVDH19.**

The internal transcribed spacer (ITS) and partial ribosomal sequences for CVDH01-CVDH19 were amplified by PCR using the primer pair ITS1 (5’-TCCGTAGGTGAACCTGCGG-3’) / NL4 (5’-GGTCCGTGTTCCTAAGACGG-3’). The sequences are listed below.
The ITS and partial ribosomal sequences for strains CVDH01-CVDH19 (the sequences were the same):

GCGGAAGGATCATTAAATAAACACTTACACACTGATTTTGACTAGTAAATAA
CCCACCAGTTAAGTTCAATTACACAATTAGTAAAAACTTTCAACAACGGGATCTTGG
TTCTCGCATCGATGAAAGACGCAGCAGAATGCGATACGTAGTATGACTTGACAGCG
TGAATCATCGAATCTTTGAACGCACATTGCGCCTTGGAGCATTCTCAATCAGGTA
GGACTACCGCTGAACTTAAGCATATCAATAAAGCGGAGGAAAAAGAAAAACAGGG
ATTGCCTCAGTAACGCGGTAGTAAGCGCAGCGGCAAGAGCTCAACTTTGGAATCGCTCCG
CGAGTTGTAGTCTGGAGGCGCCCGGCTCCGGCTTGGCAGCACCGCAACAAATCTAAGTCTCTG
GAACGAGGGCCTTGAAGGGGTAGCAGCAGCCCGGTGGATTTGTCTGTGTGCTTGGCCC
TGTTCCCTGCCGACGAGTCAGTTGTGGGGAATGCACTCAAATGGGTGTAATTT
CCATCTAAAGCTAATACCCGGGCAGAGCCGATAGCGAACAAGTACAGTAAAGGA
AGATGAAAGCCTTTGAAAAAGAGAGTAGAAGACAGTATACTGAAATTGTTGAAAGGA
AGGGCTTCAGGTAGACAAACTGTCAAATCGAGCTGAAGTGAGTGAGCTAGAAGTGCC
CTGATGTAGCAACTTCCGTTGGATTATACATCAACGGCCTGAGATAGCTCCCTG
AGGATGCTTTGGAAGGATG

DNA sequences for MLST analysis (eight genes: AAT1, ACC1, ADP1, ALA1, ERG11, RPB1, RPB2, and ZWF1)

AAT1 sequence for strains CVDH01-CVDH19:

CGTTCCAAGACCTACCAGGACCGGTCAGAAGAACTTCAATTCAAAACTTCT
GACAAGGACACCAACCGTGCTCAATTGATCAAGAGCAGCAGCGATTTGCTAGGCC
AACCATCTCCCGGTACCCGGTTCTCCCCCCGTATGAGCTCAACAGATCTAC
TCCTCGGGTCAAGATCAGTTCTACTCAAGGGAAGGTGGCTAAACACGTCGACTTGGTTC
ACCGATGCTGGTATGAAGGCTGACTTTTACGCCTACTACGACAAGGAGAACAATGGCTTGGACTTTGAGAATCTCAAGAAGTCTGTCGCTGCTGCTCCTGAGGAGTCTGTGATCTTGTTGCACGCCTGTGTCCACAAACCTACTGTTGATATGGACTTGAATCTCCCCAGGAATGGGAGGAGGTTTTGGAGATCATCCAGCAGAAGAAGCTCTTCCCTCTTGTGGACATGGCCTACCAGGGCTTCCGGTAAACACCTACGAGGACATTGGGCTTGATCA

**ACC1** sequence for strains CVDH01-CVDH19:

```
CAATGTCGAGTTGATTGTCGAAATCGCAGAGAGAACCAATGTCCACGCCGTG
TGGGCCGGCTGGGGCCACGCCTCGGAAAACCCCATTTTGGCCCGAGATGTTGGCGCCCTGCCCACAAATCGTGTTTATCGGCCCGCCAGGCTCCGCCATGAGGTCCTTGGGTGACAAGATCTCCTCCACAATCGTTGACAGCACGCCGACGTGCCCTGTATCCCCTGGTCCGGTACGGGCTGCTGGACGTTGAAATTGACAACGAAACGAAATTGGTCTCGGT
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GCAACGAGACGTCATGTTCGTGTGACGAGCTGGGAGAGAATTAACTGTAA
CATTTGTACAGATGATTCTGTTTGTGATGCTTTTATGCCCGAGGGTCTCAAGGGACTGTTACCAGCGAGGAGTTGTCATCAACGAGATTCACCAAATGTGCAATGTGACCAATCCCAAGATTATCAAGATTTTAGAGGGTGAGATTCCCCAAGCCACCTTTAGATGTGACAAAAAGAACAATACTTGTGATTTTCAATTCTGGATTGATGAGGTAGAATCTTTCTTTTGTGACTTGAGTCTGTGCAAGTTTGATTACGATCTCGAGTCGAATACAACCCGCTATAACTGTGACAATGTGGCTTGCGAGTGTTTGCCTGGACGTATGCTTTGTGCAATCTG
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GCTAACGAGATCCCTGGCTCTCCAATT
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**ADP1** sequence for strains CVDH01-12 and CVDH 14-19:

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GCAACGAGACGTCATGTTCGTGTGACGAGCTGGGAGAGAATTAACTGTAA
CATTTGTACAGATGATTCTGTTTGTGATGCTTTTATGCCCGAGGGTCTCAAGGGACTGTTACCAGCGAGGAGTTGTCATCAACGAGATTCACCAAATGTGCAATGTGACCAATCCCAAGATTATCAAGATTTTAGAGGGTGAGATTCCCCAAGCCACCTTTAGATGTGACAAAAAGAACAATACTTGTGATTTTCAATTCTGGATTGATGAGGTAGAATCTTTCTTTTGTGACTTGAGTCTGTGCAAGTTTGATTACGATCTCGAGTCGAATACAACCCGCTATAACTGTGACAATGTGGCTTGCGAGTGTTTGCCTGGACGTATGCTTTGTGCAATCTG
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GATCAAATTGATATCCTCAGAGTTTCTTGGAAAAAACTATCAAGGGTCCAGGTAGCTTTA
CTTGTTG
```
**ADP1 sequence for strains CVDH13:**

GCAACGAGACGTCATGTTGTGACGAGGGTGGGACGGAATTAACTGTAA
CATTTGTACAGATGATTCTGTGTGGATGCTTTTATGCCCCAGGGTCTCAAGGGAGTC
TGTTACCAGCGAGAGGTGTCATCAACGAGATTCCACAAATGTCGAATGTGACCAAT
CCCAAGATTATCAAGATTTTAGAGGGTGAGATTTCCCCAAGCCCACCTTTAGATGTGAC
AAAAAGAACAATACTTGTGATTTTCAATTCTGGATTGATGAGGTAGAATCTTTCTTT
GTGACTTTGAGTCTGTGCAAGTTTTGATTACGATCTCGAGTGAATACAAATTACAA
ACTGTDGACAATGTGGCTTTGCGAGTGTGTGGTACCTGGACATATGCTTTTGTGCGAAATCTG
GATCAATTGATATCTCAGAGTCTCTGAAAATAAACTATAAAGGTCAGGCTGTAGACCTTA
CTTGATG

**ALA1 sequence for strains CVDH01-19:**

TCAGAGCCGTTGGTAAGCAACAATGACTTGTGGATGAGTCCGAAAGGACTCTTTA
TCACCACACCTTTTTCGAGATGTTGGTAACTGTCTTTTGTGACTACTTCAAGAAAG
GAGGCTACTCGAGTGTCTTTGAAGAATTTGTGACTGTACCGTTCCTCCGAGAAAAAGAC
CGTTGTACGTGACCTACTTGTGAGGTGAGGTTCTGACGAGGCAGCCAGCAGACCA
GAGGCCAAGCAGTCTCAGTGATGAGCAGGGAAGCCAGGCTTTGCAAGCCAGACCCA
GACGCTTTCTGACAAACTCTCTGGAAATGGGTGATCAAGGGCACTGTGCTTCCATGTCC
GAGATCCACTAAGACAATTTGTTTGGAAAGAAACCGGCCCACTTGTGTAACATTGGA
CGACCCCATAAGTTTTGAGGGTCCTCGAGTCTTCCACGTACACACAGAGGC
AGACTCGTCTCTTATTGCTGCACTAAACAAGCAGCATTA

**ERG11 sequence for strains CVDH01-19:**

GAAGAAGTTCGCAAGACAGGCTCTGACCCAAGGAGGCTTTTCCAAAGATACGGTC
CCTAGAATCCAGAGAGGTGTGGACTACTTCAAGACCTGTGCTTGAGTACGAGAT
GAACGAGCAGACAACCGTGTGTCGACCTGATGAAGACCCAGGTGATGACCCAG
TCTTGAGCTCTTCAAGTCTTTGATGGCGACGACATGAGACAGCCAGTCTTGGATGCTT
CTTTTGCTCAGTTGTACTCCGATTTGGACAAGGGTTTCACCCCTATCAACTTTGTTTT
CCCTCACTTTGCTTTGGCCCGCTTTACTGGGAAGAGAGACGCTGCTCAGCAGAAGATCTC
GGCTACGTACATGTCTTTGATTAATGAGAGAAAGAAGTACTGGTGCACATCATCCCAAGA
CAGAGAAGTTGGATCGACTCGCCATGACCAACTCTACCTACAAGAGACGCTGCTCAGCAGA
GCTTGCACGGGTGTTGCTGTTGATGGGTGGTCAGCACAC
TTCCGCTTCCACCTC

*RBP1* sequence for strains CVDH01-19:

TGGAACGTCTGTAAGACAAAGATTTGGCTGAGCTGAGCCTCGAGCCTCAACGATG
AAAGCCAGTTACCTCTGGGAAGAGCCGCTGCTGTCACACACACACACTCTACCTGCTA
GAGATGGTATAGGTGTTGGGAAACATGGAACAAACACAGTGGAGGAAA
CGAACAGCCGGAGCCTGCTTGGTTACCCATCGGAGATTTTGATGTTTTTCAGACA
CATCAGTAAGAAAGACTGTCAAGAGTTGGGCTTCAATGAAGACTATGCAAGACCAG
AGTGGATGTGTGTACACCGTTCTACCTCTGTCACCCCCACCTGTGAAGCACCCTCAGATTG
CTTCAACGATACTTGCTAGAGGTGAAGATGAAGTTGACTGACATTTAAGTTGGCTGATATCA
TCAAGCCAAATGCAACGGACTCGAAATGGACGGTTCTCCTCAGCAGTTA
TCAGTGAGTTGAAAGCTTCTTTTACAGTT

*RPB2* sequence for strains CVDH01-19:

TGAGGATGCCCAGACCGAAAGGTATTTTTGGTGTAAGGTGCTATCATGTTTGGCTT
CCAAGTTCTGATGTTGCGGTAGCTTGGGCGAAGACAGTTCTACGAGTTGAAAGGAGT
GCCCATACGATATGGGTGTTACTTTGTCATCAACGGTTCCGAGAAGGTTTTGATTG
CCCAGGAGCTTCTGCTGCTGCTAATATTGTGAAGGTCTTACACCCACTCGGAGGCCCC
CTATTTCCACGTGGCCAGATCAAGATCCCGCCCTCCGAGAAGGTTCTCACGGTGTGATCT
CCTCCATGCAAATCAGGGTTAGAGAGACGACAAGGGCACCCTCCCGCAGAAC
ATCAAGGGCTACCTTGCCATACATCAAGAAGACACATCCCTATCGTTATCGTTTTTAGA
GCCCTCGGTGTTTGTCCCTGATGTTATCCTTGGAGCACATTTGTTACGACGCTAAT
GACTGGCAAATGTTGGAGATGT

ZWF1 sequence for strains CVDH01-19:

GAAGAGTGAGAGTCATTGTCGAGAAGCCCTTCGGCCACGATTTGGAGTCTTC
CAGACAATTGCAGAAAGATTTGGCTCCTCTTTTCACTGAGGAAGAATTGTACAGAAT
TGACCACCTACTTGGGCAAGGAATGTGAAGAAACTGTTGGTGTTGCGGTATTGGTAA
TGAGTTATGGTCGTCGTGGTGAACAACAGGCTATATTTCCTCGGTCCAGATTTTCCTTT
AAAGAGGCATTGGAACACAGAGGAAGAGGGCGGTACTTTTGACCTGATCGGCAATAAT
CAGAGACGTCACTGCAAGAACCACCTATTTAGGCTAGGTGGTGACCTTTGGACCATTGGAGAG
ACCTGTGTCGTTGACCACGAGGTCTGAGAGATGAAAAGTGAAGGTGCTCAAGG
CTTTTGACGATTTTACCCCAACGACATCTTGGCTCGGTCAATATGGTAAGTCTGAAG
ATGGCTCTAAGCC

References

1. Huang G, Yi S, Sahni N, Daniels KJ, Srikantha T, Soll DR. N-acetylglucosamine induces white to

zinc finger transcription factor, in biofilm formation, filamentous growth and virulence. PLoS

2013;2013:923742.

Reclassification of the Candida haemulonii complex as Candida haemulonii (C. haemulonii group
I), C. duobushaemulonii sp. nov. (C. haemulonii group II), and C. haemulonii var. vulnera var.


Appendix Table. Detailed information on the patients with C. vulturna infections*

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age, y</th>
<th>Diagnosis</th>
<th>Facility type</th>
<th>Specimen source</th>
<th>Strain collection date (Days after admission)</th>
<th>Time of admission, Days</th>
<th>Patient outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Male</td>
<td>45</td>
<td>Hypertension (stage III) and Brain surgery</td>
<td>Neuroscience ward</td>
<td>Blood</td>
<td>Jan 17, 2019 (16 days)</td>
<td>Jan 1, 2019 – Feb 12, 2019 (42 days)</td>
<td>Routine discharge</td>
</tr>
<tr>
<td>C2</td>
<td>Male</td>
<td>60</td>
<td>Traumatic subarachnoid hemorrhage tSAH, intraventricular hemorrhage, scalp laceration, traumatic pneumonia</td>
<td>Neuroscience ward</td>
<td>Blood</td>
<td>Jun 20, 2019 (20 days)</td>
<td>May 31, 2019 – Sep 11, 2019 (103 days)</td>
<td>Routine discharge</td>
</tr>
<tr>
<td>C3</td>
<td>Male</td>
<td>57</td>
<td>Chronic bronchitis, pulmonary infection</td>
<td>ICU</td>
<td>Blood</td>
<td>Aug 3, 2019 (81 days)</td>
<td>May 14, 2019 – Aug 8, 2019 (86 days)</td>
<td>Discontinued care</td>
</tr>
<tr>
<td>C4</td>
<td>Male</td>
<td>73</td>
<td>Chronic cough and expectoration, Hypertension (stage III)</td>
<td>ICU</td>
<td>Blood</td>
<td>Aug 13, 2019 (3 days)</td>
<td>Aug 10, 2019 – Sep 2, 2019 (23 days)</td>
<td>Discontinued care</td>
</tr>
<tr>
<td>C5</td>
<td>Male</td>
<td>43</td>
<td>Injuries to the spleen, rib fractures</td>
<td>General surgery ward</td>
<td>Blood</td>
<td>Aug 16, 2019 (38 days)</td>
<td>Jul 9, 2019 – Nov 16, 2019 (130 days)</td>
<td>Routine discharge/ self care</td>
</tr>
<tr>
<td>C6</td>
<td>Male</td>
<td>78</td>
<td>Acute abdominal disease, Hypertension (stage I)</td>
<td>ICU and PICC tip</td>
<td>Blood†</td>
<td>Aug 18, 2019 (13 days)</td>
<td>Aug 5, 2019 – Sep 21, 2019 (47 days)</td>
<td>Routine discharge</td>
</tr>
<tr>
<td>C7</td>
<td>Female</td>
<td>16</td>
<td>Injuries to the head, face are, chest and abdominal tissues caused by car accident</td>
<td>ICU</td>
<td>Blood</td>
<td>Aug 27, 2019 (7 days)</td>
<td>Aug 20, 2019 – Dec 26, 2019 (128 days)</td>
<td>Routine discharge/ self care</td>
</tr>
<tr>
<td>C8</td>
<td>Male</td>
<td>73</td>
<td>Thoraco-abdominal and pelvic inj</td>
<td>ICU and Neuroscience ward</td>
<td>Blood</td>
<td>Sep 21, 2019 (14 days)</td>
<td>Sep 7, 2019 – Nov 6, 2019 (60 days)</td>
<td>Routine discharge/ self care</td>
</tr>
<tr>
<td>Case</td>
<td>Sex</td>
<td>Age, y</td>
<td>Diagnosis</td>
<td>Facility type</td>
<td>Specimen source</td>
<td>Strain collection date (Days after admission)</td>
<td>Time of admission, Days</td>
<td>Patient outcome</td>
</tr>
<tr>
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<tr>
<td>C10</td>
<td>Male</td>
<td>66</td>
<td>Trigeminal neuralgia, hypertension</td>
<td>ICU</td>
<td>Blood† and PICC tip</td>
<td>Oct 12, 2019 (73 days)</td>
<td>Jul 19, 2019 – Nov 14, 220 (118 days)</td>
<td>Routine discharge</td>
</tr>
<tr>
<td>C11</td>
<td>Female</td>
<td>13</td>
<td>Serious intracranial injury, multiple tissue injuries caused by car accident</td>
<td>ICU</td>
<td>Blood† and PICC tip</td>
<td>Nov 1, 2019 (11 days)</td>
<td>Oct 21, 2019 – Jan 13, 2020 (84 days)</td>
<td>Routine discharge</td>
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<tr>
<td>C12</td>
<td>Male</td>
<td>71</td>
<td>Bile duct cancer</td>
<td>General surgery ward</td>
<td>PICC tip</td>
<td>Nov 19, 2019 (30 days)</td>
<td>Oct 20, 2019 – Dec 13, 2019 (54 days)</td>
<td>Routine discharge</td>
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<tr>
<td>C13</td>
<td>Male</td>
<td>78</td>
<td>Periodic fever</td>
<td>General Medicine ward</td>
<td>Blood</td>
<td>Dec 28, 2019 (32 days)</td>
<td>Nov 26, 2019 – Jan 20, 2020 (55 days)</td>
<td>Routine discharge</td>
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<tr>
<td>C14</td>
<td>Male</td>
<td>83</td>
<td>Chronic obstructive pulmonary disease, lower respiratory tract infection, hypertension (stage III)</td>
<td>ICU</td>
<td>PICC tip</td>
<td>Sep 18, 2020 (19 days)</td>
<td>Aug 30, 2020 – Sep 22, 2019 (23 days)</td>
<td>Discontinued care</td>
</tr>
<tr>
<td>C16</td>
<td>Male</td>
<td>63</td>
<td>Consciousness Disorder, Septic shock, sepsis, pulmonary and urinary tract infections</td>
<td>ICU</td>
<td>Blood</td>
<td>Feb 11, 2022 (43 days)</td>
<td>Dec 30, 2021 – Mar 6, 2022 (66 days)</td>
<td>Expired</td>
</tr>
<tr>
<td>C17</td>
<td>Male</td>
<td>57</td>
<td>Injuries to the head caused by car accident</td>
<td>ICU</td>
<td>PICC tip</td>
<td>Jun 30, 2022 (35 days)</td>
<td>May 26, 2022 – Jul 13, 2022 (48 days)</td>
<td>Routine discharge</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age, y</th>
<th>Diagnosis</th>
<th>Facility type</th>
<th>Specimen source</th>
<th>Strain collection date (Days after admission)</th>
<th>Time of admission, Days</th>
<th>Patient outcome</th>
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<tbody>
<tr>
<td>C18</td>
<td>Male</td>
<td>66</td>
<td>Advanced gastric cancer, bladder cancer</td>
<td>Internal Medicine - Oncology</td>
<td>Blood† and PICC tip</td>
<td>Jul 24, 2022 (17 days)</td>
<td>Jul 7, 2022 – Aug 30, 2022 (54 days)</td>
<td>Routine discharge</td>
</tr>
<tr>
<td>C19</td>
<td>Male</td>
<td>60</td>
<td>Intracranial infection</td>
<td>Neuroscience ward</td>
<td>Blood</td>
<td>Aug 26, 2022 (32 days)</td>
<td>Jul 25, 2022 – Oct 26, 2022 (94 days)</td>
<td>Routine discharge</td>
</tr>
</tbody>
</table>

*PICC, peripherally inserted central catheter; ICU, intensive care unit.
†When *C. vulturna* was isolated from two or more specimen sources, strains isolated from the blood samples were used for biological and DNA sequencing analyses.

**Appendix Figure 1.** Monthly incidence of *C. vulturna* infections in a Shanxi, China hospital. C1–C19, patient cases 1 to 19. Only two cases of *C. vulturna* infections were found during the peak COVID-19 period, January 1, 2020–January 1, 2022, perhaps because of the enhanced hygiene measures implemented.
Appendix Figure 2. Maximum-likelihood phylogeny analysis of the CTG clade species based on the internal transcribed spacer and partial ribosomal sequences. The tree was generated using the program RAxML (https://cme.h-its.org/exelixis/web/software/raxml). The general time reversible model model, gamma distribution, 1,000 bootstraps, and midpoint root were adopted. Strain sequence information: C. haemulonii (CBS10969, JX459773.1), C. haemulonii var. vulneris (CBS12439, MK394151.1), C. pseudohaemulonii (CBS10004, MK394152.1), C. duobushaemulonii (CBS7798, MK394153.1) from the NCBI GeneBank; SRR11091965–67, SRR11092032, SRR11092036, and SRR22996287 from the NCBI WGS database; C. auris (B8441), Clavispora lusitaniae (ATCC42720), C. parapsilosis (CDC317), C. orthopsilosis (Co90–125), C. tropicalis (MYA3404), C. albicans (SC5314), C. dubliniensis (CD36) from the CTG database (http://www.candidagenome.org).
Appendix Figure 3. Biofilm morphologies of *C. vulturna* and *C. auris* isolates. *C. vulturna* strains are CVDH01, CVDH03-05, CVDH07-17, and CVDH19. *C. auris* strains are BJCA001 and SJ-01. Biofilms were developed on silicone squares at 30°C for 24 hours. Lee’s glucose medium was used for biofilm growth. Compared to the other *C. vulturna* isolates, strains CVDH07, CVDH12, and CVDH13 developed weaker biofilms on the silicone squares. *C. auris* strain SJ01 developed robust biofilms, whereas *C. auris* strain BJCA001 formed comparatively weaker biofilms. This figure is associated with Figure 2 in the main article.