

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

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Candida vulturna belongs to the *Candida haemulonii* species complex and is phylogenetically related to *C. auris*. We report a *C. vulturna* outbreak among persons in Shanxi Province, China, during 2019–2022. Isolates were resistant to multiple antifungal drugs and exhibited enhanced adhesion and biofilm formation properties.

Candida vulturna, a fungal pathogen that is phylogenetically related to *C. haemulonii* and *C. auris*, was isolated from flowers in a taxonomic study of yeasts in 2016 (1,2). Since then, *C. vulturna* has been sporadically isolated in different countries from clinical specimens such as blood, wounds, and peripherally inserted central catheters (PICCs) (1–4). *C. vulturna*, *C. haemulonii*, and *C. auris* belong to the *Metschnikowia/Candida* clade (1,5). Antifungal drug resistance, especially to the azoles, is a common feature of species within this clade. During 2009–2022, fungal infections caused by the reportedly rare species *C. haemulonii* and *C. auris* have become more prevalent in clinical settings (1,6–10). The increased occurrence of those infections could be the result of the widespread use of antifungal agents in clinical and agricultural settings, as well as the environmental changes caused by human activities (10–12).

In China, reports of infections caused by the superbug fungus *C. auris* have been relatively infrequent; however, the prevalence of *C. haemulonii* and associated species in the *C. haemulonii* complex has been steadily increasing in recent years (8,13). For

our study, we analyzed deidentified health records of patients infected with *C. vulturna*, as approved by the ethics committee of a general hospital in Shanxi Province, China.

The Study

We selected a total of 19 patients, 17 male and 2 female, who had been infected with *C. vulturna* during January 1, 2019–October 26, 2022 (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/29/7/23-0254-App1.pdf>). We isolated 16 *C. vulturna* strains directly from the blood through venipuncture and 7 strains from a PICC line tip of the 19 patients (Appendix Table). We initially identified the strains as *C. haemulonii* complex species by growth on CHROMagar *Candida* medium (CHROMagar, <https://www.chromagar.com>) and confirmed by sequencing of the ribosomal internal transcribed spacer (ITS) region. Most cases were identified in 2019; *C. vulturna* infections were identified in 2 patients during January 1, 2020–January 1, 2022. Enhanced hygiene measures taken at that time may have dampened the spread of *C. vulturna* in the hospital.

On the basis of results of the ITS and multilocus sequence typing for 8 conserved genes, we then performed phylogenetic analyses on the isolates. All strains isolated in this study (CVDH01–19) were closely related by phylogenetic analyses and clustered together in 1 clade (Figure 1; Appendix Figure 2).

The hospital has 1 intensive care unit (ICU). Of the 19 patients we identified as infected with *C. vulturna*, 11 were from the ICU, 4 were from the neuroscience ward, and 4 were from other departments within the hospital. The age range of patients was 13–83 years (median 63 years). Because all patients

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had PICC lines for delivery of medications and *C. vulturna* strains were isolated from the PICC line tips of 7 patients, the use of PICC lines could be a major risk factor for *C. vulturna* infection. Other risk factors could include traumatic injuries, hypertension, cancer, and blood and pulmonary infections (Appendix Table). We also conducted environmental screening assays but were unable to detect or isolate *C. vulturna* from hospital surfaces, including walls, floors, bedside tables, bed sheets, bed frames, blood pressure cuffs, and chairs.

We used 1 representative *C. vulturna* strain from each patient for subsequent antifungal drug

susceptibility testing and phenotypic analyses (Appendix Table). Using the breakpoints established for *C. albicans*, we determined that all 19 of the *C. vulturna* strains tested were resistant to azole drugs (Table). All isolates were resistant to amphotericin B (MIC 4 mg/L) but were susceptible to echinocandins (MICs ≤ 0.125 for caspofungin, ≤ 0.125 for anidulafungin, ≤ 0.5 for micafungin), and flucytosine (MIC 0.06).

When grown in liquid media, we observed that the cells from the *C. vulturna* (CVDH) strains isolated in this study formed large aggregates and exhibited enhanced adhesion and biofilm formation abilities. This feature was similar to that of *C. auris* strain SJ01, which formed enhanced biofilms under both in vitro and in vivo conditions (14). (Figure 2; Appendix Figure 3).

Conclusion

A serious threat to human health is the emergence of new multidrug-resistant fungal species. Both the widespread use of antifungal agents and the reduced susceptibility of these emerging species to antifungal drugs could contribute to the epidemiologic shifts toward multidrug-resistant fungal pathogens that we are increasingly observing in clinical settings. In this study, we report an outbreak of *C. vulturna*, which is phylogenetically closely related to *C. haemulonii* and *C. auris*, in a general hospital in Shanxi Province, China. We observed that the implementation of general enhanced hygiene measures remarkably decreased overall infection rates during the COVID-19 pandemic period (January 1, 2020–January 1, 2022) in this hospital; our findings suggest that the transmission of *C. vulturna* may be preventable through enhanced disinfection methods. Most of the *C. vulturna* isolates we obtained were from patients with bloodstream infections, defined as a single isolation of *C. vulturna* from blood obtained through venipuncture. Phylogenetic analyses indicated that the outbreak strains were closely related (Figure 1; Appendix Figure 2), implying that those strains could have originated from the same ancestor.

Striking characteristics of the *C. vulturna* strains isolated in this study were their enhanced adhesion and biofilm formation abilities. It is conceivable that those characteristics may be key contributors in promoting the spread of *C. vulturna* strains between patients during this outbreak. Consistent with this hypothesis, we observed that the use of PICC lines was a critical risk factor for *C. vulturna* infections. Another notable characteristic of the *C. vulturna* strains isolated in this study was their reduced susceptibilities to azole drugs and amphotericin B (Table), which has

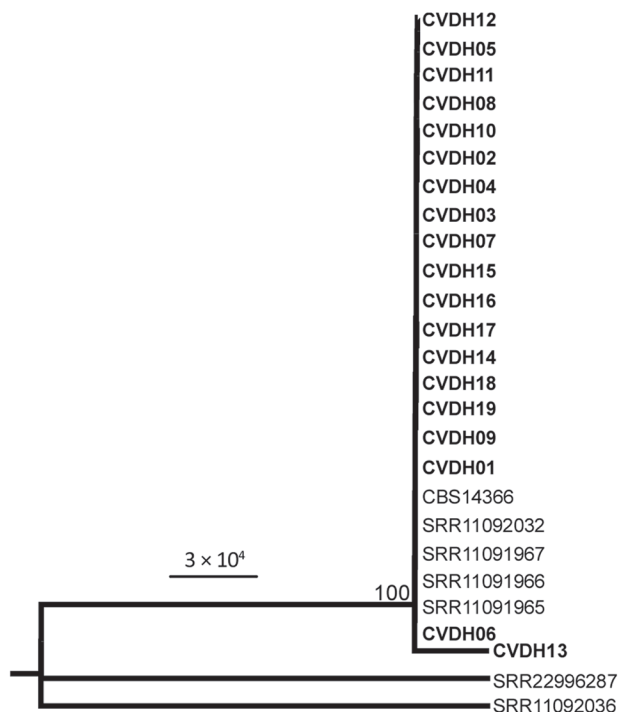


Figure 1. Maximum-likelihood phylogeny analysis of *Candida vulturna* strains from 19 infected patients in Shanxi Province, China, January 1, 2019–October 26, 2022, based on multilocus sequence typing (MLST). Eight genes (*AAT1*, *ACC1*, *ADP1*, *ALA1*, *ERG11*, *RPB1*, *RPB2*, and *ZWF1*) were concatenated and used for phylogenetic analyses. The tree was generated using the program RAXML (<https://cme.h-its.org/exelixis/web/software/raxml>). The general time reversible model, gamma distribution, 1,000 bootstraps, and midpoint root were adopted. Bold text indicates strains isolated in this study; reference strain data from whole-genome sequencing is from the National Center for Biotechnology Information gene database (accession nos. SRR11091965–67, SRR11092032, SRR11092036, SRR22996287). Sequences for strain CBS14366 were retrieved from its genomic assembly (GenBank accession no. GCA_026585945.1). Strains CVDH01–CVDH19 were isolated from patients of *C. vulturna* infection (cases C1–C19; Table; Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/29/7/23-0254-App1.pdf>). Scale bar indicates substitutions per site.

Table. Susceptibility profiles of *Candida vulturna* isolates from 19 infected patients to 9 antifungal drugs, Shanxi Province, China, January 1, 2019–October 26, 2022*

Patient no.	Strain ID	FLC	VOC	ITC	POC	CAS	MFG	AFG	5-FC	AMB
C1	CVDH01	32	32	64	64	0.125	0.5	0.125	0.06	4
C2	CVDH02	128	32	32	16	0.06	0.5	0.125	0.06	4
C3	CVDH03	64	32	32	32	0.06	0.25	0.125	0.06	4
C4	CVDH04	128	32	16	16	0.125	0.5	0.06	0.06	4
C5	CVDH05	128	32	32	32	0.125	0.5	0.06	0.06	4
C6	CVDH06	128	32	32	32	0.125	0.5	0.125	0.06	4
C7	CVDH07	256	64	64	64	0.06	0.25	0.125	0.06	4
C8	CVDH08	128	32	32	32	0.25	0.5	0.125	0.06	4
C9	CVDH09	128	32	32	64	0.06	0.25	0.25	0.06	4
C10	CVDH10	128	32	16	16	0.125	0.5	0.125	0.06	4
C11	CVDH11	256	64	64	64	0.125	0.5	0.125	0.06	4
C12	CVDH12	128	32	64	64	0.06	0.25	0.125	0.06	4
C13	CVDH13	64	32	32	32	0.06	0.25	0.125	0.06	4
C14	CVDH14	64	16	32	32	0.03	0.5	0.03	0.06	4
C15	CVDH15	128	64	32	16	0.06	0.25	0.125	0.06	4
C16	CVDH16	128	32	64	32	0.125	0.5	0.125	0.06	4
C17	CVDH17	64	32	32	32	0.06	0.5	0.06	0.06	4
C18	CVDH18	64	8	32	32	0.06	0.5	0.06	0.06	4
C19	CVDH19	64	32	32	32	0.06	0.5	0.06	0.06	4

*MIC assays were performed according to Clinical and Laboratory Standards Institute microdilution guidelines. Bold text indicates antifungal resistance (based on the breakpoints for *C. albicans*). AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; FLC, fluconazole; ITC, itraconazole; MFG, micafungin; POC, posaconazole; VOC, voriconazole; 5-FC, flucytosine.

also been observed in other species of the *C. haemulonii* complex (6,7,13).

The occurrence of infections caused by fungal species of the *Metschnikowia* clade has become more and more frequent in clinical settings, especially during 2009–2022 (1,6,8,13). The widespread use of antifungal drugs in clinical settings and fungicides

in agricultural settings could be contributors to the increased emergence of these multidrug resistant fungal pathogens. Given the transmissible, adhesive, and antifungal drug-resistant characteristics of emerging *C. vulturna* clinical isolates, *C. vulturna* could be a serious upcoming threat to hospital infections worldwide.

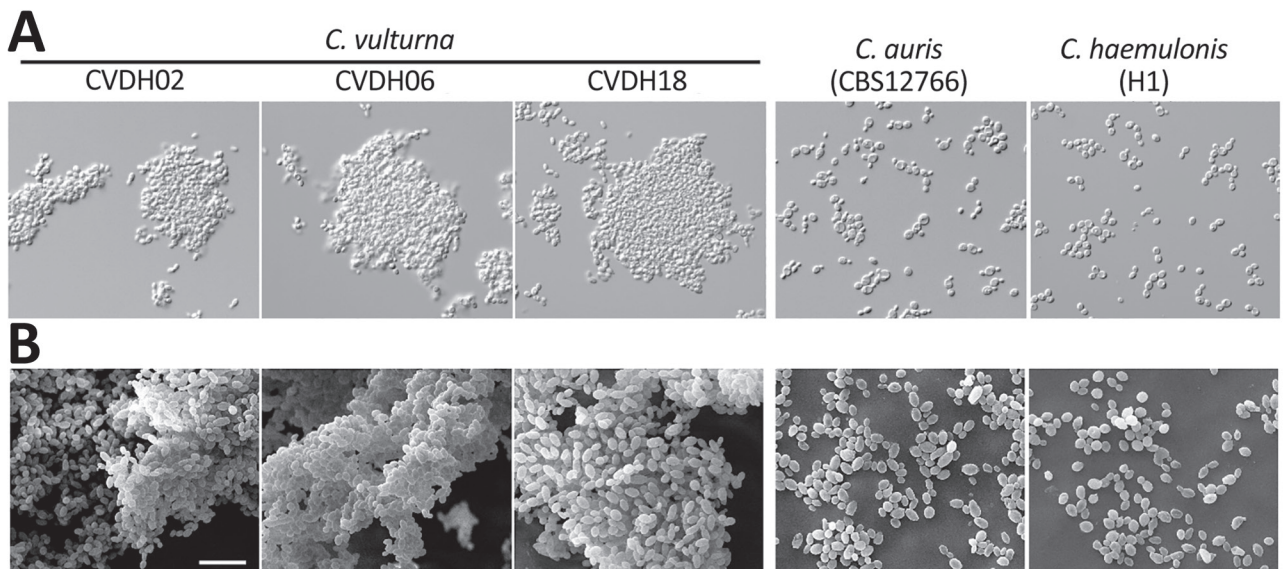


Figure 2. Morphologies of 3 representative *C. vulturna* isolates from 19 infected patients in Shanxi Province, China, January 1, 2019–October 26, 2022. *C. auris* (CBS12766) and *C. haemulonii* (H1) served as reference strains. A) Adhesion phenotypes of *C. vulturna* isolates grown in liquid Lee's glucose medium at 30°C for 24 h. Strains CVDH02, CVDH06, and CVDH18 exhibited strong adhesiveness, whereas the *C. auris* and *C. haemulonii* reference strains grew as separate single cells under the same culture conditions. B) Biofilm formation of *C. vulturna* isolates. *C. auris* (CBS12766) and *C. haemulonii* (G7) served as reference strains. Biofilms were developed on silicone squares at 30°C for 48 h. Lee's glucose medium was used for biofilm growth. Scale bar indicates 10 μm. Morphologies for the other 16 *C. vulturna* isolates and 2 *C. auris* strains are shown in Appendix Figure 3 (<https://wwwnc.cdc.gov/EID/article/29/7/23-0254-App1.pdf>).

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C.J.N. is a cofounder of BioSynthesis, Inc., a company developing diagnostics and therapeutics for biofilm infections.

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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 OD₆₀₀ 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 2×10^6 cells of each strain were inoculated into each well containing one silicone square (10 mm x 10 mm, Bentec Medical, INC., Woodland) and 600 μ L Lee's glucose medium. After incubation for 48 hours at 30°C with shaking, the silicone squares were washed gently with ddH₂O 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*, *ALAI*, *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: aaggagtacacgggtatcac, AAT1R: aacgagctcgttcaatcttc

ACC1 (515 bp):

CvACC1F: accaacaacaacaactacgc, CvACC1R: ccagccaacttcatgatgaa

ADP1 (499 bp):

ADP1F: ttcaaaaagaccaccagag, ADP1R: acactctccaccgtaatgt

ALA1 (530 bp):

ALA1F: tgcgaactcccaaaagtga, *ALA1R*: tttcaaaaccataccggtg

ERG11 (562 bp):

ERG11F: aactctcgtttgatggagca, *ERG11R*: aatgcaacaagaaccaagca

RPB1 (516 bp):

RPB1F: agaagagatttaatgcggtg, *RPB1R*: ccatgtatgtagcaacgtga

RPB2 (513 bp):

RPB2F: atcgaggagaaggtggagaa, *RPB2R*: ttctcaacacagggttca

ZWF1 (503 bp):

ZWF1F: actcgtctatcctgaaggtg, *CvZWF1R*: tctcgtcgtcaaggtagat

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'-GGTCCGTGTTTCAAGACGG-3'). The sequences are listed below.

The ITS and partial ribosomal sequences for strains CVDH01-CVDH19 (the sequences were the same):

GCGGAAGGATCATTTAAAATAACACTTACACACTGATTTTGACTAGTAAATAA
CCCACCAGTTAAGTTCAATTACACAATTAGTAAAACCTTCAACAACGGATCTCTTGG
TTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTATGACTTGCAGACG
TGAATCATCGAATCTTTGAACGCACATTGCGCCTTGGAGCATTCTCCAAGGCATGCC
TGTTTGAGCGTGATTTCTTCTCACCGCACCCGGTGGTTTTGCATCCGCGCTAAATATC
ATTCCAGCAGCGAAGTCTACGCTTTCACTGCTCCATGCTATTTCTCAAATCAGGTA
GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGG
ATTGCCTCAGTAACGGCGAGTGAAGCGGCAAGAGCTCAACTTTGGAATCGCTCCGG
CGAGTTGTAGTCTGGAGGCGGCCGGTCCGCCTTGCGCAACCAAATCTAAGTCCTCTG
GAACGAGGCGCTTGAAAGGGTGACAGCCCCGTGGATTTGTCTGTTGTGCTTGGCCCC
TGGTCCTGCCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGTGGTAAATT
CCATCTAAAGCTAAATACCGGCGAGAGACCGATAGCGAACAAGTACAGTAATGGAA
AGATGAAAAGCACTTTGAAAAGAGAGTGAAACAGTACGTGAAATTGTTGAAAGGGA
AGGGCTTGCAGGTAGACAACACTGTCAGCATCGGGTGGAGTGGAGCTAGAAGTGGGCG
CTGATGTAGCAACTTCGGTTGCATTATACAACGCTCAGATAGCTCCCGTTTCGCCCCG
AGGATCGCCTTTTGAAGGATG

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

AAT1 sequence for strains CVDH01-CVDH19:

CGGTTCCAAGACCTACCAGGACGCGGTCAAGAACTTCATTTTCAACAACCTCT
GACAAGGACACCAACGGTGCTCAATTGATCAAGGACGGCCGTATTGTCACTGCCCA
AACCATCTCCGGTACCGGTTCTCTCCGTGTTATCGCCGATTTCTCAACAGATTCTAC
TCCTCGGGTCAGATCATCGTTCCTAAGCCAACCTGGGCTAACCACGTCGCTGTGTTC

ACCGATGCTGGTATGAAGGCTGACTTTTACGCCTACTACGACAAGGAGAACAATGG
CTTGACTTTGAGAATCTCAAGAAGTCTGTCGCTGCTGCTCCTGAGGAGTCTGTGAT
CTTGTTGCACGCCTGTTGCCACAACCCTACTGGTATGGACTTGACTCCCCAGGAATG
GGAGGAGGTTTTGGAGATCATCCAGCAGAAGAAGCTCTTCCCTCTTGTGGACATGGC
CTACCAGGGCTTCGCCTCCGGTAACACCTACGAGGACATTGGCTTGATCA

ACC1 sequence for strains CVDH01-CVDH19:

CAATGTCGAGTTGATTGTCGAAATCGCAGAGAGAACCAATGTCCACGCCGTG
TGGGCCGGCTGGGGCCACGCCTCGGAAAACCCCATTTTGCCCGAGATGTTGGCCGCC
CTGCCCAAAAAAATCGTGTTTATCGGCCCGCCAGGCTCCGCCATGAGGTCCTTGGGT
GACAAGATCTCCTCCACAATCGTTGCACAGCACGCCGACGTGCCCTGTATCCCCTGG
TCCGGTACGGGCGTGCTGGACGTTGAAATTGACAACGAAACGAAATTGGTCTCGGT
GTCCGAAGAGACTTACGCCAAGGGCTGCTGCACGAGTCCGGAAGACGGCTTGAAAA
AAGCCCGCCAGATCGGTTTCCCGTCATGATCAAGGCCTCCGAGGGTGGAGGCGGT
AAAGGTATCCGTAAGGTCGACAACGAGGACGATTTTATCTCCTTGTAACAAGCAGGCT
GCTAACGAGATCCCTGGCTCTCCAATT

ADP1 sequence for strains CVDH01-12 and CVDH 14-19:

GCAACGAGACGTCATGTTCGTGTGACGACGGTTGGGACGGAATTAAGTGTAA
CATTTGTACAGATGATTCTGTTTGTGATGCTTTTATGCCCCGAGGGTCTCAAGGGAGTC
TGTTACCAGCGAGGAGTTGTCATCAACGAGATTCACCAAATGTGCAATGTGACCAAT
CCCAAGATTATCAAGATTTTAGAGGGTGAGATTCCECAAGCCACCTTTAGATGTGAC
AAAAAGAACAATACTTGTGATTTTCAATTCTGGATTGATGAGGTAGAATCTTTCTTTT
GTGACTTGAGTCTGTGCAAGTTTGTATTACGATCTCGAGTCGAATACAACCCGCTATA
ACTGTGACAATGTGGCTTGCAGAGTGTTCCTGGACGTATGCTTTGTGGCAAATCTG
GATCAATTGATATCTCAGAGTTCCTTGAAAAAACTATCAAGGGTCCAGGTGACTTTA
CTTGTG

ADP1 sequence for strains CVDH13:

GCAACGAGACGTCATGTTTCGTGTGACGACGGTTGGGACGGAATTAAGTGTAA
CATTGTACAGATGATTCTGTTTGTGATGCTTTTATGCCCCGAGGGTCTCAAGGGAGTC
TGTTACCAGCGAGGAGTTGTCATCAACGAGATTCACCAAATGTGCAATGTGACCAAT
CCCAAGATTATCAAGATTTTAGAGGGTGAGATTCCCCAAGCCACCTTTAGATGTGAC
AAAAAGAACAATACTTGTGATTTTCAATTCTGGATTGATGAGGTAGAATCTTTCTTTT
GTGACTTGAGTCTGTGCAAGTTTGATTACGATCTCGAGTCGAATACAACCCGCTATA
ACTGTGACAATGTGGCTTGCAGAGTGTTCCTGGACGTATGCTTTGTGGCAAATCTG
GATCAATTGATATCTCAGAGTTCCTTGAAAAAACTATCAAGGGTCCAGGTGACCTTA
CTTGTG

ALA1 sequence for strains CVDH01-19:

TCAGAGCCGGTGGTAAGCACAATGACTTGGATGACGTCGGAAAGGACTCTTA
TCACCACACCTTTTTTCGAGATGTTGGGTAAGTGGTCTTTTGGTGACTACTTCAAGAAG
GAGGCTATCGAGTGGTCTTGGGAATTGTTGACTGAGGTCTTCAAGTTGGAAAAAGAC
CGTTTGTACGTGACCTACTTTGAGGGTGACGAGAAGAACGGCTTGCAGCCAGACCTA
GAGGCCAAGCAGTTCTGGTTGGATGTCGGTGTGGCCGAGGACCACATCTTGCCGGGT
GACGCTTCTGACAACTTCTGGGAAATGGGTGATCAAGGCCCATGTGGTCCATGTTCC
GAGATCCACTACGACAGAATTGGTGGAAAGAAACGCCGCCCACTTGGTGAACATGGA
CGACCCCAATGTTTTGGAGGTCTGGAACGTCGTGTTTCATTCAGTACAACAGAGAGGC
AGACTCGTCTCTTAGGCCATTGCCTAACAAGCACATTGA

ERG11 sequence for strains CVDH01-19:

GAAGAAGTTCGCAAAGACAGCCTTGACCAAGGAAGCTTTCCAAAGATACGTC
CCTAGAATCCAGGAGGAGGTGTTGGACTACTTCAAGACCTGTCCTGAGTACGAGAT
GAACGAGCGCAACAACGGTGTGGCAACGTGATGAAGACCCAGCCTGAGATGACCA
TCTTGACTGCTTCCAAGTCTTTGATGGGCGACGACATGAGAGCCAAGTTTGATGCCT

CTTTTGCTCAGTTGTACTCCGATTTGGACAAGGGTTTCACCCCTATCAACTTTGTTTT
CCCTCACTTGCCTTTGCCCGCTTACTGGAAGAGAGACGCTGCTCAGCAGAAGATCTC
GGCTACGTACATGTCCTTGATTAATGAGAGAAGAAGTACTGGTGACATCATCCCAGA
CAGAGACTTGATCGACTCGCTCATGACCAACTCTACCTACAAAGACGGTGTCAAGAT
GACCGACCAGGAGGTTGCCAACTTGTTGATCGGTGTCTTGATGGGTGGTCAGCACAC
TTCCGCTTCCACCTC

RBP1 sequence for strains CVDH01-19:

TGGAACGTCTGTAAGACAAAGATGGTCTGTGAAGCTGACGTCGTCAACGATG
AAGGCCAGGTTACCTCTGGAAGAGGGCGGCTGTGGTCACACACAACCTACTGTTTCGTA
GAGATGGTATGAAGTTGTGGGGAACATGGAAACAGAACAAACAGTTTGAGGAAAA
CGAACAGCCCGAGCGTCGTTTGTGACCCCATCGGAGATTTTGAGTGTTTTTCAGACA
CATCAGTGAAGAAGACTGTCAGAAGTTGGGCTTCAATGAAGACTATGCAAGACCAG
AGTGGATGTTGATCACCGTTCTACCTGTTCCACCCCCACCTGTGAGACCCTCGATTG
CTTCAACGATACTGCTAGAGGTGAGGATGACTTGACATTTAAGTTGGCTGATATCA
TCAAAGCCAATATCAACGTTCAACGACTCGAAATGGACGGTTCTCCTCAGCACGTTA
TCAGTGAGTTTGAAGCTCTTTTACAGTT

RPB2 sequence for strains CVDH01-19:

TGAGGATGCCCAGACCAAGGTATTTTTGGGTAAGGTGCCTATCATGTTGCGTT
CCAAGTTCTGTATGTTGCGTGACTTGGGCGAACACGAGTTCTACGAGTTGAAGGAGT
GCCCATACGATATGGGTGGTTACTTTGTCATCAACGGTTCCGAGAAGGTTTTGATTG
CCCAGGAGCGTTCTGCTGCTAATATTGTGCAAGTCTTCAAGAAGGCGGCGCCTTCCC
CTATTTCCACGTTGCCGAGATCAGATCCGCCCTCGAGAAGGGTTCACGGTTGATCT
CCTCCATGCAAATCAAGTTGTACGGTAGAGACGACAAGGGCACTTCCGGCAGAACC
ATCAAGGCTACCTTGCCATACATCAAAGAAGACATCCCTATCGTTATCGTTTTTTAGA

GCCCTCGGTGTTGTCCCTGATGGTGATATCTTGGAGCACATTTGTTACGACGCTAAT
GACTGGCAAATGTTGGAGATGT

ZWF1 sequence for strains CVDH01-19:

GAAGAGTGAGAGTCATTGTGCGAGAAGCCCTTCGGCCACGATTTGGAGTCTTC
CAGACAATTGCAGAAAGATTTGGCTCCTCTTTTCACTGAGGAAGAATTGTACAGAAT
TGACCACTACTTGGGCAAGGAAATGGTGAAGAACTTGTTGGTGTTCGTTTTGGTAA
TGAGTTATGGTCTGGTGTGTGGAACAACAGGCATATTTCCCTCGGTCCAGATTTCCCTT
AAAGAGGCATTCGGAACAGAGGGAAGAGGGCGGCTACTTTGACCTGATCGGCATAAT
CAGAGACGTCATGCAGAACCACTTATTGCAGGTGTTGACCCTTTTGACCATGGAGAG
ACCTGTGTCGTTTCGACCCAGAGGCTGTGAGAGATGAAAAAGTGAAGGTGCTCAAGG
CTTTTGACGATTTTAACCCCAACGACATCTTGCTCGGTCAATATGGTAAGTCTGAAG
ATGGCTCTAAGCC

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

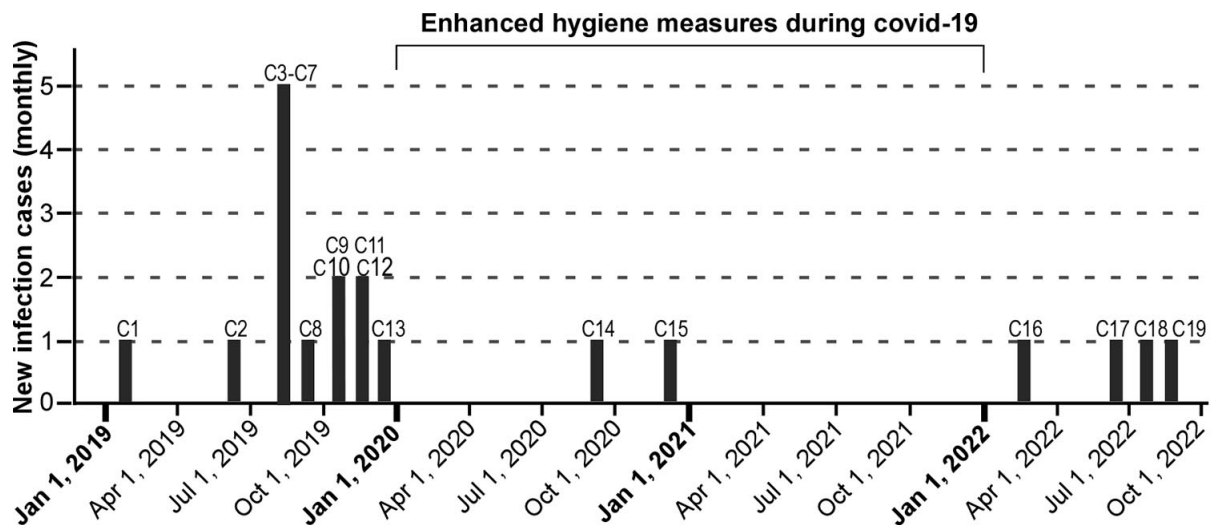
Case	Sex	Age, y	Diagnosis	Facility type	Specimen source	Strain collection date (Days after admission)	Time of admission, Days	Patient outcome
C1	Male	45	Hypertension (stage III) and Brain surgery	Neuroscience ward	Blood	Jan 17, 2019 (16 days)	Jan 1, 2019 – Feb 12, 2019 (42 days)	Routine discharge
C2	Male	60	Traumatic subarachnoid hemorrhage tSAH, intraventricular hemorrhage, scalp laceration, traumatic pneumonia	Neuroscience ward	Blood	Jun 20, 2019 (20 days)	May 31, 2019 - Sep.11, 2019 (103 days)	Routine discharge
C3	Male	57	Chronic bronchitis, pulmonary infection	ICU	Blood	Aug 3, 2019 (81 days)	May 14, 2019 – Aug 8, 2019 (86 days)	Discontinued care
C4	Male	73	Chronic cough and expectoration, Hypertension (stage III)	ICU	Blood	Aug 13, 2019 (3 days)	Aug 10, 2019 – Sep 2, 2019 (23 days)	Discontinued care
C5	Male	43	Injuries to the spleen, rib fractures	General surgery ward	Blood	Aug 16, 2019 (38 days)	Jul 9, 2019 – Nov 16, 2019 (130 days)	Routine discharge/self care
C6	Male	78	Acute abdominal disease, Hypertension (stage I)	ICU	Blood† and PICC tip	Aug 18, 2019 (13 days)	Aug 5, 2019 – Sep 21, 2019 (47 days)	Routine discharge
C7	Female	16	Injuries to the head, face are, chest and abdominal tissues caused by car accident	ICU	Blood	Aug 27, 2019 (7 days)	Aug 20, 2019 – Dec 26, 2019 (128 days)	Routine discharge/self care
C8	Male	73	Thoraco-abdominal and pelvic inj	ICU and Neuroscience ward	Blood	Sep 21, 2019 (14 days)	Sep 7, 2019 – Nov 6, 2019 (60 days)	Routine discharge/self care

Case	Sex	Age, y	Diagnosis	Facility type	Specimen source	Strain collection date (Days after admission)	Time of admission, Days	Patient outcome
			uries caused by car accident					
C9	Male	46	Traumatic epidural hematoma, scalp injury and skull fracture	ICU	Blood	Oct 11, 2019 (9 days)	Oct 2, 2019 – Jan 14, 2020 (104 days)	Routine discharge/ self care
C10	Male	66	Trigeminal neuralgia, hypertension	ICU	Blood† and PICC tip	Oct 12, 2019 (73 days)	Jul 19, 2019 – Nov 14, 220 (118 days)	Routine discharge
C11	Female	13	Serious intracranial injury, multiple tissue injuries caused by car accident	ICU	Blood† and PICC tip	Nov 1, 2019 (11 days)	Oct 21, 2019 – Jan 13, 2020 (84 days)	Routine discharge
C12	Male	71	Bile duct cancer	General surgery ward	PICC tip	Nov 19, 2019 (30 days)	Oct 20, 2019 – Dec 13, 2019 (54 days)	Routine discharge
C13	Male	78	Periodic fever	General Medicine ward	Blood	Dec 28, 2019 (32 days)	Nov 26, 2019 – Jan 20, 2020 (55 days)	Routine discharge
C14	Male	83	Chronic obstructive pulmonary disease, lower respiratory tract infection, hypertension (stage III)	ICU	PICC tip	Sep 18, 2020 (19 days)	Aug 30, 2020 – Sep 22, 2019 (23 days)	Discontinued care
C15	Male	66	Paraplegia, type 2 diabetes, pulmonary and urinary tract infections	ICU	Blood	Nov 18, 2020 (19 days)	Oct 30, 2020 – Dec 15, 2020 (46 days)	Discontinued care
C16	Male	63	Consciousness Disorder, Septic shock, sepsis, pulmonary and urinary tract infections	ICU	Blood	Feb 11, 2022 (43 days)	Dec 30, 2021 – Mar 6, 2022 (66 days)	Expired
C17	Male	57	Injuries to the head caused by car accident	ICU	PICC tip	Jun 30, 2022 (35 days)	May 26, 2022 – Jul 13, 2022 (48 days)	Routine discharge

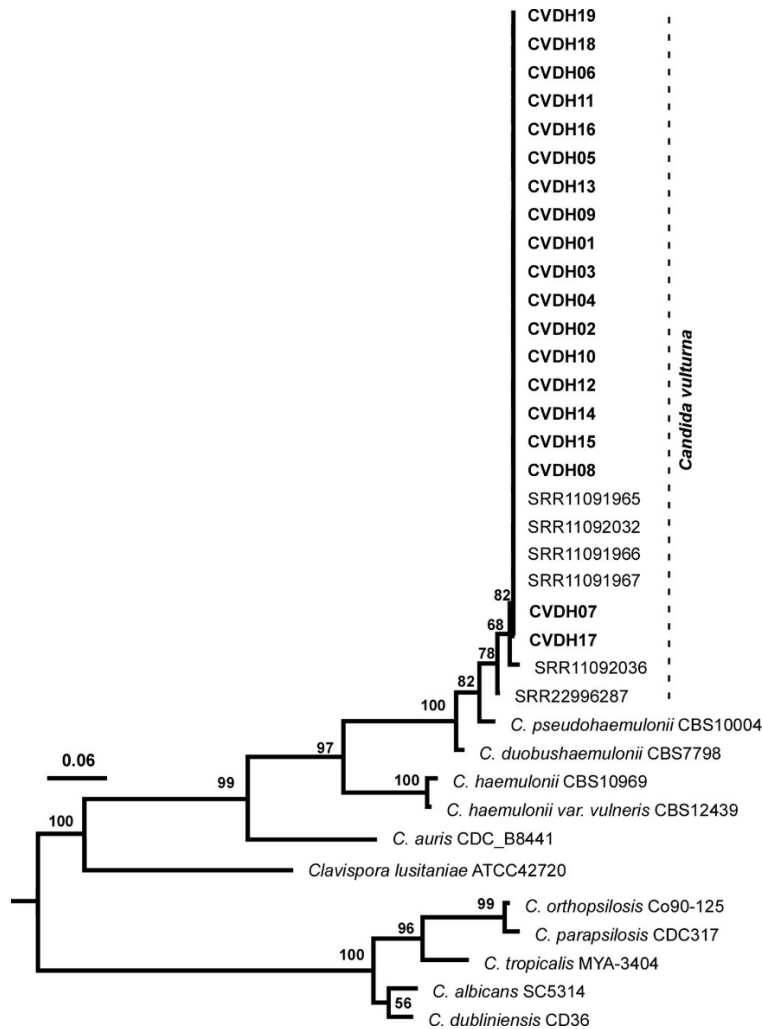
Case	Sex	Age, y	Diagnosis	Facility type	Specimen source	Strain collection date (Days after admission)	Time of admission, Days	Patient outcome
C18	Male	66	Advanced gastric cancer, bladder cancer	Internal Medicine - Oncology	Blood† and PICC tip	Jul 24, 2022 (17 days)	Jul 7, 2022 – Aug 30, 2022 (54 days)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

*PICC, peripherally inserted central catheter; ICU, intensive care unit.

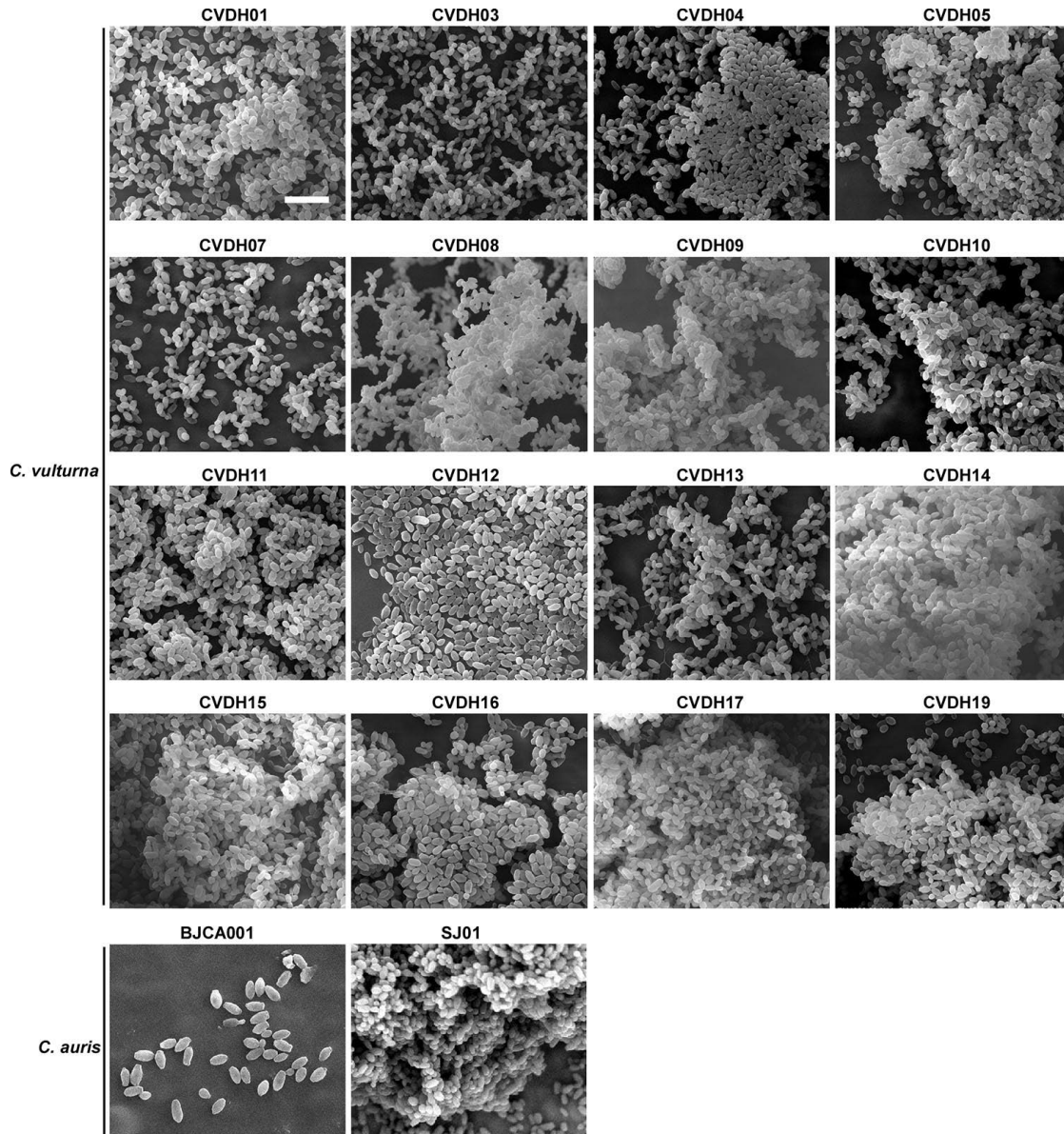
†When *C. vulturna* was isolated from two or more specimen sources, strains isolated from the blood samples were used for biological and DNA sequencing analyses.



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. pseudoaemulonii* (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 BJA001 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 BJA001 formed comparatively weaker biofilms. This figure is associated with Figure 2 in the main article.