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ORIGINAL RESEARCH

Convergence of Hypervirulence and Multidrug-Resistance in *Burkholderia cepacia* Complex Isolates from Patients with COVID-19

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Purpose: *Burkholderia* is a conditioned pathogen in the medical setting and mainly affects patients with cystic fibrosis. We found co-infection with *Burkholderia cepacia* complex (Bcc) in many patients with respiratory tract infections, including H7N9 and COVID-19. However, previous studies have not focused on co-infections with BCC and respiratory viruses. Therefore, this study attempted to clarify the evolution of COVID-19-Bcc and H7N9-Bcc in terms of genetic background, antibiotic resistance, and virulence phenotypes.

Methods: This study retrospectively collected 49 Bcc isolated from patients with H7N9 and COVID-19 in a tertiary hospital of Zhejiang Province, of which 42 isolates were isolated from patients with H7N9, seven isolates were isolated from patients with COVID-19. The collected isolates were tested for antibiotic susceptibility, *Galleria mellonella* infection model, and whole-genome COVID-19-Bcc Characterization.

Results: The test results of 49 strains of Bcc showed that the strains isolated from COVID-19 patients accounted for 57.1% of multidrug-resistance resistant strains. Statistical analysis of the median lethal time of *G. mellonella* showed that the median fatal time for COVID-19-Bcc was shorter and more virulent than that of H7N9-Bcc ($P<0.05$). The results of phylogenetic analysis indicated that COVID-19-Bcc may have evolved from H7N9-Bcc.

Conclusion: In this study, co-infection with BCC in many patients with respiratory tract infections, including H7N9 and COVID-19, was first identified and clarified that COVID-19-Bcc may have evolved from H7N9-Bcc and has the characteristics of hypervirulence and multidrug resistance.

Keywords: *Burkholderia cepacia* complex, COVID-19, H7N9, hypervirulence, multidrug-resistance, comparative genomic analysis

Introduction

The new coronavirus member "Severe Acute Respiratory Syndrome Coronavirus 2" SARS-CoV-2 causing the Coronavirus Disease 2019 (COVID-19) as per the World Health Organization (WHO), is one of the highly pathogenic β-coronaviruses which infect humans and have caused a pandemic worldwide.[1] The early clinical manifestations and spread of SARS-CoV-2 are similar to influenza.[2] Similarly, the pandemic caused by human infection with avian influenza A (H7N9) virus, like SARS-CoV-2, has caused significant morbidity and extremely high mortality in humans.[2] However, respiratory viral infections are well known to predispose patients to bacterial co-infections and superinfections.[3] According to reports, bacterial co-infections in patients with severe influenza are as high as 20–30%.[4] Bacterial co-infection forces patients with viral infections to consume more medical resources and may cause more serious diseases, thereby increasing the mortality rate of patients.[5]

The genus *Burkholderia* is composed of Gram-negative β-proteobacteria, which are widely found in water, soil, and plant rhizosphere.[6] *Burkholderia* has diverse metabolism and strong adaptability, growing in resistant environments and becoming conditional pathogens in medical environments.[4] The primary *Burkholderia* species that cause human

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infections are the members of the *Burkholderia cepacia* complex (Bcc) and *Burkholderia pseudomallei*.[7] Bcc includes *Burkholderia cepacia, Burkholderia multivorans*, etc.[8] It primarily impacts patients with cystic fibrosis and ranks after ESCAPE as a primary pathogen in clinical treatment.[9] The highly pathogenic Bcc has caused outbreaks in many regions of the world.[10,11] Infections caused by these bacteria are difficult to treat due to significant antibiotic resistance.[12,13] However, few studies have focused on the impact of Bcc on virus-infected patients.

In hospitals and ICU, the reported coinfection rate of viruses and bacteria can reach up to 68% and significantly affect patient mortality. Existing reports on SARS-CoV-2 co-infection with bacteria have focused on the types and proportions of bacteria that cause the infection. However, there have not been studies focused on the genetic background of bacteria, antibiotic resistance, virulence phenotype, and the impact on patients with viral infections from the perspective of the genome.[8] In this study, we found that SARS-CoV-2 and BCC co-infection occurred in some COVID-19 patients, and this phenomenon also occurred in H7N9 patients. Therefore, we retrospectively collected BCC isolates from patients with H7N9 and COVID-19 at a tertiary hospital in Zhejiang Province from 2013 to 2020. This study grouped and compared the susceptibility, virulence phenotype, and genomic characteristics of the isolates, and clarified the hypervirulence and multidrug resistance of BCC isolates from patients with COVID-19.

Materials and Methods

Sample Collection and Identification

This study retrospectively collected 49 BCC isolates from patients with H7N9 and COVID-19 in a tertiary hospital in Zhejiang Province from 2013 to 2020, of which 42 were recovered from patients with H7N9, seven isolates from patients with COVID-19. In addition, to eliminate the natural evolutionary factors of bacteria, Bcc isolated from other patients in 2020 were randomly selected as a reference. The experimental strains are all part of the routine laboratory process.

The accurate identification of Bcc is questionable by conventional biochemical methods.[8] Therefore, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany) was used to identify bacteria. In addition, high-throughput ANI analysis was used to compare the whole-genome sequencing results to reveal clear species of Bcc,[14] with the reference strains of *B. cepacia* (GCF001718895) and *B. multivorans* (GCF000959525).

Antibiotic Susceptibility Testing

The minimum inhibitory concentrations (MICs) of antibiotics (Dalian Meilun Biotech Co., Ltd., Dalian, China) were determined using the agar dilution method.[15] Antibiotics used for testing included meropenem, ceftazidime, minocycline, levofloxacin, trimethoprim-sulfamethoxazole, and chloramphenicol. Test results were interpreted using CLSI standards (https://clsi.org). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains.

Survival Rates of Galleria mellonella Larvae Infected with Bcc

BCC strains were inoculated on blood agar and cultured overnight at 37°C in a thermostatic incubator. The BCC strain was resuspended in NaCl solution and the optical density at 600 nm was 1.0. Ten *G. mellonella* weighing approximately 250 mg were randomly divided into groups, and each *G. mellonella* was injected with 20μL of 1×10^7 CFU/mL bacteria. The negative control group was injected with only 20μL PBS. All *G. mellonella* were cultured at 37 °C, and the status of *G. mellonella* was observed every 12 h for seven consecutive days.[16] *E. coli* ATCC 25922 was used as the quality control strain.

Biofilm Quantification

Luria-Bertani (LB, Sangon Biotech, China) overnight cultures were collected, washed, and adjusted to an OD 600 of 0.1 LB. A pipette to take 200μL bacterial suspension and drop it into 96-well polystyrene microplates (Corning). Each sample was repeated thrice and incubated at 37°C for 24 h. Subsequently, the wells were washed three times with PBS (0.1 mol/L, pH 7.4), incubated with 100 μL of 0.1% crystal violet stain, and incubated at room temperature for 15 min. After washing the wells with PBS three times, 200 μL isopropanol was injected and gently shaken, and the absorbance of the sample solution was measured at OD600 after mixing.[16]

Growth Assay

BCC strains were cultured overnight in Mueller-Hinton broth (MHB) medium (Cat. No. CM0405; OXOID, UK) were normalized to OD600 (0.02 (1×10^5 CFU/mL) in the same medium and grown at 37°C for 18 h with vigorous aeration (220 rpm). Drop 180μL of MHB was added to a 96-well polystyrene microplate, and then add 20μL 1×10^5 CFU/mL bacterial suspension was added. The cell density was determined every hour by measuring the OD600.[16] The test lasted 40 h in total.

Whole-Genome Sequencing (WGS) and Data Analysis

Genomic DNA was extracted using an OMEGA Bacterial DNA Kit (Omega Bio-tek, Norcross, GA, USA). WGS was performed using an Illumina NovaSeq 6000-PE150 platform (Illumina, San Diego, CA, USA). Use SPAdes 3.11 to combine Illumina sequencing reads into the genome sequence. Antimicrobial resistance genes (http://www.genomicepidemiology.org/), virulence genes (http://www.mgc.ac.cn/VFs/), and MLST (http://pubmlst.org/) were compared using the online databases.

Phylogenetic Reconstruction and Analysis

Using *B. multivorans* (GCF_000959525.1) as a reference sequence,[17] GATK software (GATK (broadinstitute.org)) was used to analyze the nucleotide polymorphisms. The alignment file was filtered from variants with elevated densities of base substitutions as putative recombination events by Gubbins version 2.4.1.[18] The filtered core-genome alignment file was used to construct a maximum likelihood tree using FastTree with the GTR+CAT model.[19] Use PHYLOViZ 2.0 (http://www.phyloviz.net/tutorials.html) to generate a minimum spanning tree for cgSNPs.[20] The 49 Bcc strains in this study were compared with the data in the NCBI database. A maximum-likelihood tree of the core SNP matrix output of kSNP[21] was generated using iTOL (https://itol.embl.de/). The CARD database (https://card.mcmaster.ca/) was used to compare the efflux pump genes carried by the bacteria.

Statistical Analysis

Genome-wide association study (GWAS) method was used in R Studio software and the Kruskal–Wallis Test was selected to compare whether there was a statistical difference between the 3 groups of data. A P-value of 0.05 was used as the level of significance; $P<0.05$ indicated statistically significant differences.

Data Availability

The whole-genome sequences of the 49 Bcc were submitted to GenBank under the accession numbers JAGXEV000000000-JAGXCZ000000000.

Results

Identification Result of Bcc

The study population included H7N9 patients hospitalized in a tertiary hospital in Zhejiang Province between 2013 and 2014 and COVID-19 patients hospitalized between 2019 and 2020. A total of 49 Bcc strains were collected: 42 isolates were isolated from H7N9 patients, and seven isolates were isolated from COVID-19 patients. The results of MALDI-TOF/MS and ANI analysis showed that among 49 Bcc, 21 were *B. multivorans*, and 28 were *B. cepacia* (Figure 1).

Molecular Characteristics of 14 BCC

The data for the antimicrobial resistance genes are shown in Figure 2. Only two *B. multivorans* strains were compared for aminoglycoside resistance genes. The virulence-related genes detected in BCC included flagella-related, chemotaxis-related, type IV fimbriae-related, N-acyl homoserine lactone-dependent, and capsular polysaccharide-related genes (Figure 2). A database was used to compare the ST types of the strains (Figure 2). There was no matching ST type for *B. cepacia* and ST1008 (57.1%) was the main ST type for *B. multivorans*. Additionally, ST274 (4.8%), ST618 (4.8%), ST16 (9.5%), ST616 (9.5%), and ST749 (9.5%) were compared in *B. multivorans*.

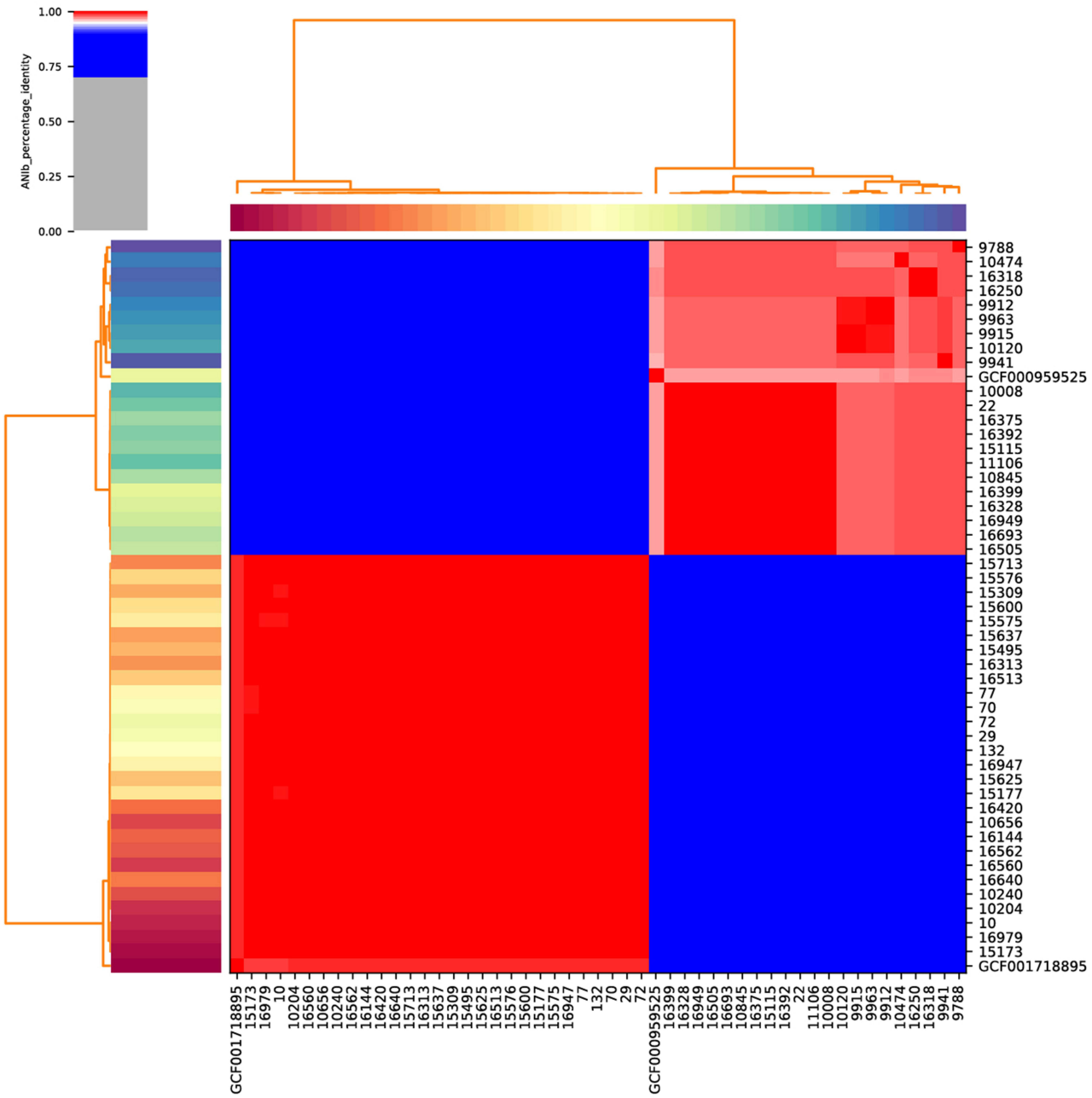

Figure 1 ANI analysis results of 49 BCC isolates. *B. cepacia* (GCF001718895) was used as the reference strain for comparison to identify *B. cepacia*. The red part of the horizontal axis in the figure is the compared *B. cepacia*. *B. multivorans* (GCF000959525) was used as the reference strain for comparison to identify *B. multivorans*. The blue part of the horizontal axis in the figure is the compared *B. multivorans*..

Antibiotic Resistance Profiles of 49 BCC

The results of antibiotic susceptibility tests are shown in Figure 2. The meropenem resistance rates in the H7N9 and COVID-19 strains were 0% and 14.3%, respectively, while those of ceftazidime were 7% and 28.6%, respectively; minocycline resistance rates were 2.3% and 85.7%, levofloxacin resistance rates were 52.4% and 71.4%, trimethoprim-sulfamethoxazole resistance rates were 0% and 0%, and chloramphenicol resistance rates were 0% and 42.9%, respectively. The strains isolated from H7N9 patients were not multidrug-resistance, whereas those isolated from COVID-19 patients accounted for 57.1% of the multidrug-resistance resistant strains. The experimental results of 30 reference isolates showed that bacterial resistance to levofloxacin was as high as 70% and resistance to Minocycline and

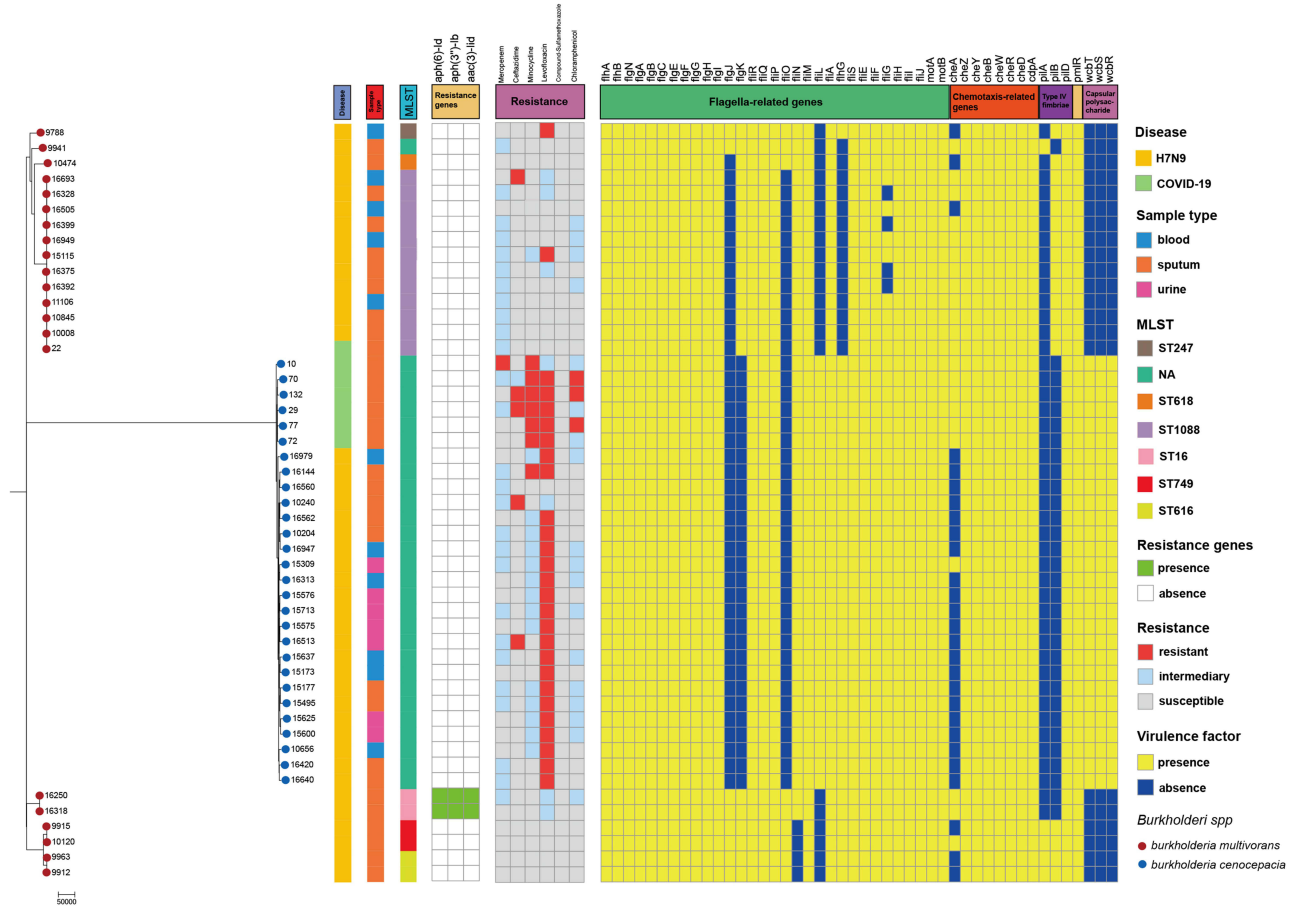

Figure 2 Construction of phylogenetic trees of *B. cepacia* and *B. multivorans* isolates. The information in the figure includes the sample source of the isolate, MLST, antibiotic resistance gene, antibiotic susceptibility test, and the comparison result of virulence gene.

Trimethoprim-sulfamethoxazole was as low as 0%. Only 3 of the 30 bacterial isolates of bacteria are multidrug resistant strains (Supplementary Material 1).

Growth Kinetics

To compare the in vitro growth rates of different BCC strains, the growth kinetics were determined for each strain at 37°C in MH broth (Figure 3). The doubling times of the isolates were grouped and compared, and the results showed no difference in growth between the two groups (P=0.16, P>0.05).

G. mellonella Infection Model

In this study, a *G. mellonella* infection model was used for in vivo analysis to verify bacterial virulence. We compared the median lethal time of *G. mellonella* infected with the H7N9 and COVID-19 BCC strains to assess the difference in pathogenicity of the strains. The results are shown in Figure 4. Statistical analysis of the median lethal time of *G. mellonella* showed that the median lethal time of COVID-19-Bcc was shorter and more virulent (P=0.00041, P<0.05). In addition, we compared the median lethal time of COVID-19-Bcc with that of 30 reference strains, and the results showed that the median lethal time of COVID-19-Bcc was shorter and more virulent (P=0.00041, P<0.05, Supplementary Material 2).

Comparison of Biofilm Formation Ability

Bacterial biofilms constitute a critical problem in hospitals, especially in resuscitation units or for immunocompromised patients, so we compared the difference in the ability of strains from H7N9 patients with those from COVID-19 patients to form biofilms.[22]

https://doi.org/10.2147/IDR.S495676

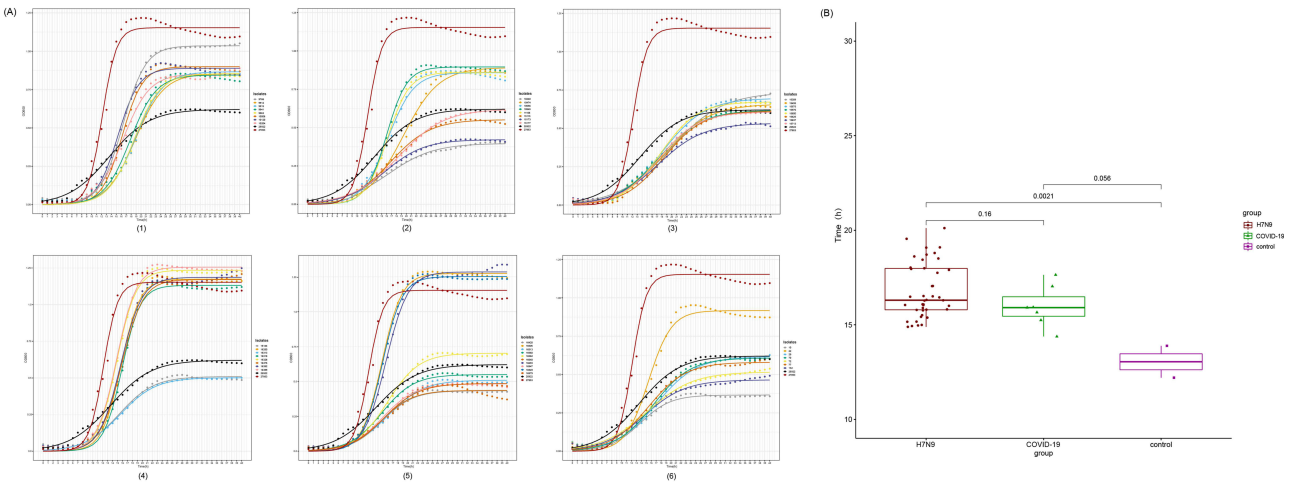

Figure 3 Growth kinetics results of *B. cepacia* and *B. multivorans* isolates. (**A**). (1)- (5) are the growth kinetic results of H7N9-Bcc, and (6) are the growth kinetic results of COVID-19-Bcc. (**B**). H7N9-Bcc, COVID-19-Bcc, the statistical results of the control group, P<0.05, is considered statistically significant.

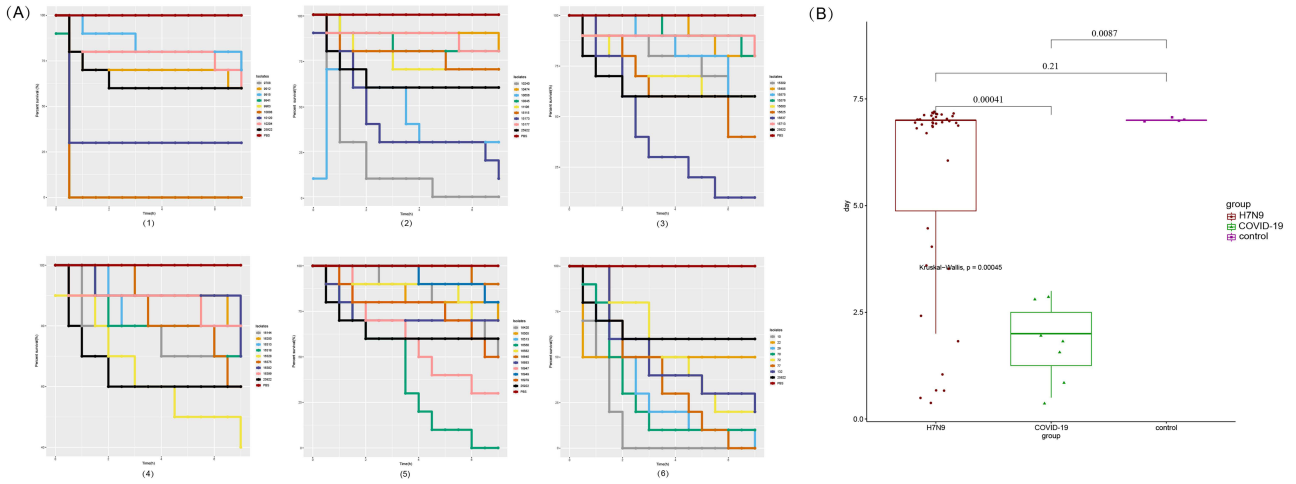

Figure 4 The virulence phenotype of *B. cepacia* and *B. multivorans* isolates. (**A**). The survival time of *B. cepacia* and *B. multivorans* infected with *G.mellonella*. (1)- (5) is the result of H7N9-Bcc infection with *G.mellonella*. (6) It is the result of COVID-19-Bcc infection with *G.mellonella*. (**B**). H7N9-Bcc, COVID-19-Bcc, the statistical results of the control group, P<0.05, are considered statistically significant.

After using isopropanol to dissolve the biofilm, the OD600 value was measured, and group statistics were performed. The results showed no difference in biofilm formation between the two groups (P=0.084, P>0.05). The results are shown in Figure 5.

Phylogeny Analysis

A phylogenetic tree was constructed based on the SNPs. The results showed that *B. cepacia* and *B. multivorans* were located in different branches and strains of the same ST type clustered together. In *B. cepacia*, the strain from the COVID-19 patient is a branch, and the strain from the same patient with the same sample type was a branch (Figure 2). On the other hand, to study the evolutionary history of 49 Bcc and identify potential ancestral hosts and geographic reservoirs, kSNP3 was used for phylogenetic reconstruction. In this study, all *B. multivorans* and *B. cepacia* isolates from NCBI 2000 were selected for phylogenetic analyses. Analysis of *B. multivorans* showed 156 isolates from clinical samples (88.1%) and 21 isolates from environmental samples (11.9%). Analysis of *B. cepacia* revealed 50 isolates from clinical samples, accounting for 88.1%, including 28 strains of *B. cepacia* in this study (Figure 6).

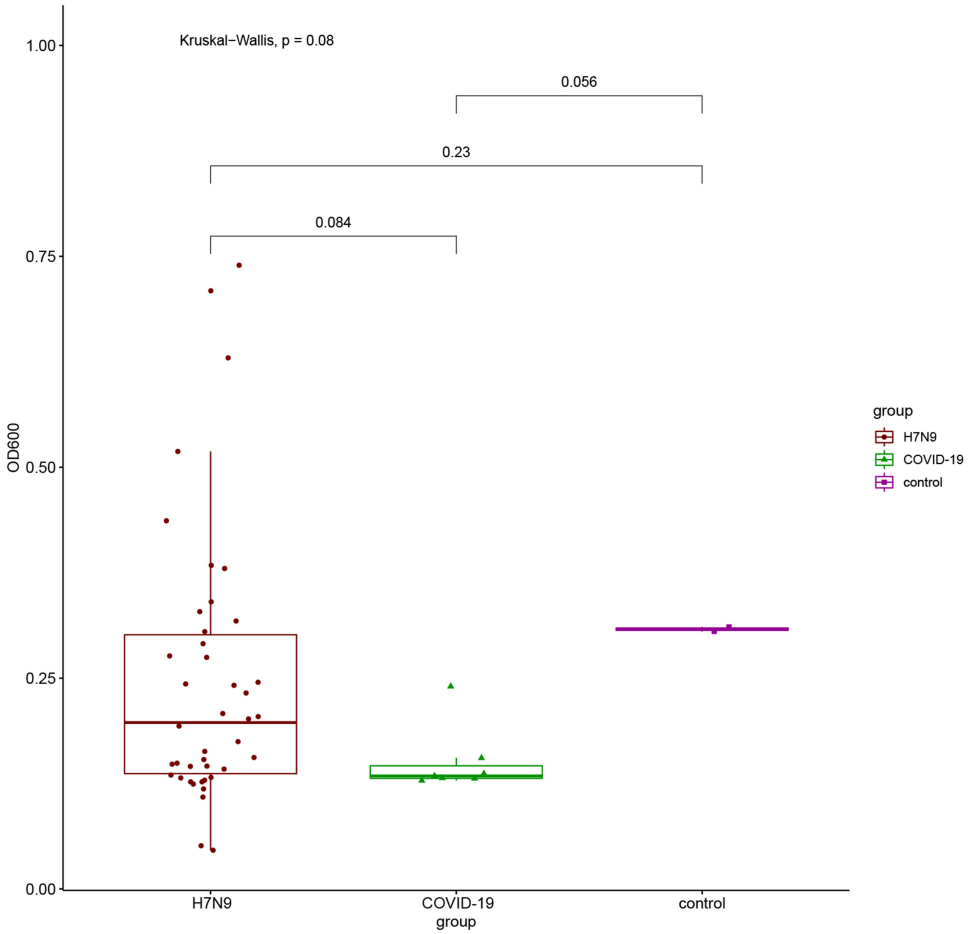

Figure 5 The biofilm formation ability of *B. cepacia* and *B. multivorans* isolates. H7N9-Bcc, COVID-19-Bcc, the statistical results of the control group, P<0.05, is considered statistically significant.

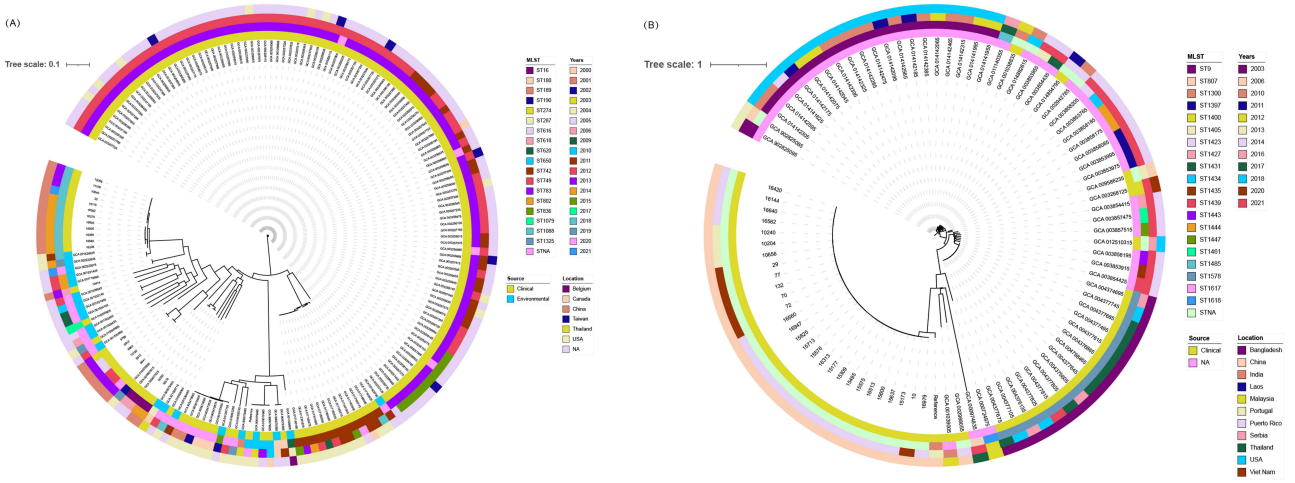

Figure 6 (**A**). Phylogenetic analysis of 177 *B. multivorans*. The circle from the inside to the outside once represents the source of the sample, the ST type, the year of isolation of the isolate, and the location. (**B**). Phylogenetic analysis of 92 *B. cepacia*. The circle from the inside to the outside once represents the source of the sample, the ST type, the year of isolation of the isolate, and the location.

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Discussion

Coinfection with viruses and bacteria constantly threatens the lives of patients. However, no studies have focused on the genetic background of bacteria, antibiotic resistance, virulence phenotype, or the impact on patients with viral infections from a genomic perspective. Through retrospective analysis, we found that SARS-CoV-2 and BCC were co-infected in COVID-19 patients, and the same situation occurred in H7N9 patients. Therefore, this study retrospectively collected 49 isolates from Bcc co-infected COVID-19 patients and H7N9, using whole-genome sequencing for the first time, and clarifying that COVID-19-Bcc may have evolved from H7N9-Bcc and has the characteristics of hypervirulence and multidrug resistance. This study selected the six antibiotics mentioned in the CLSI to analyze BCC resistance. The results of this study showed that the resistance rate of COVID-19-Bcc was higher than that of H7N9-Bcc. The antibiotic with the highest resistance rate in H7N9-Bcc was levofloxacin (52.4%), and the resistance rates of the other drugs were between 0% and 7%. The antibiotics with the highest resistance rate in COVID-19-Bcc were minocycline and levofloxacin (85.7% and 71.4%, respectively). The proportion of multidrug-resistance strains in COVID-19-Bcc is 57.1%, while H7N9-Bcc is 0%, indicating the multidrug-resistance characteristics of COVID-19-Bcc.[23] Both COVID-19-Bcc and H7N9-Bcc have 0% resistance to trimethoprim-sulfamethoxazole, proving that trimethoprim-sulfamethoxazole can effectively treat Bcc infection.[24]

B. multivorans and *B. cepacia*, belonging to the genus *Burkholderia* are prominent pathogens. Infections caused by Bcc are difficult to treat because of their apparent antibiotic resistance.[25] Only two *B. multivorans* of 49 Bcc have detected quinolone antibiotic resistance genes. Two isolates of *B. multivorans* 16250 and 16318, carrying resistance genes, were isolated from a 62-year-old female H7N9 patient. None of the remaining 47 BCC harbored resistance genes. The antibiotic resistance mechanism of BCC has been intensively studied, and its main resistance mechanism is the overexpression of efflux pumps. As in other non-enteric Gram-negatives, efflux pumps of the resistance nodulation cell division (RND) family are the significant efflux systems in Bcc.[26] Efflux pump overexpression can enable bacteria to acquire antibiotic resistance genes such as glycosides, chloramphenicol, fluoroquinolones and tetracyclines.[18]

BCC is an opportunistic pathogen that can cause persistent respiratory infections in patients with cystic fibrosis, which poses great challenges to clinical treatment.[18] Many members of the genus *Burkholderia* have significant virulence potential.[25,27] In this study, we used the *G. mellonella* infection model to compare the virulence potential of COVID-19-Bcc and H7N9-Bcc. The *G. mellonella* infection model has been widely used to identify the virulence of multiple bacteria, and is also widely used in *Burkholderia* research.[20] As shown in Figure 3, the median lethal time of COVID-19-Bcc was shorter than H7N9-Bcc. The results showed that the virulence of COVID-19-Bcc was stronger than that of H7N9-Bcc. The virulence of bacteria mainly depends on the invasive enzyme adhesin and bacterial secretion system. The colonization of hypervirulent strains makes patients more prone to invasive infections, which aggravates their condition and poses great challenges to clinical treatment.[28] Bcc can form biofilms on abiotic surfaces (such as glass and plastic) and biotic surfaces (such as epithelial cells), which play an important role in the persistent infection of Bcc.[29] The formation of biofilm is closely related to the virulence of bacteria,[30] which can promote the colonization of bacteria in different environments and protect bacteria against harmful substances such as host lysozyme and complement factors.[31] The biofilm formation ability results show no significant difference between COVIDD-19-Bcc and H7N9-Bcc (Figure 4). In addition, we compared the growth kinetics of bacteria, and the results showed that there was no significant difference between the growth kinetics of COVID-19-Bcc and H7N9-Bcc (Figure 5). It can be seen that the virulence of COVID-19-Bcc is enhanced, but the formation of biofilm and the growth rate have not changed. Therefore, we compared the virulence genes carried in COVID-19-Bcc and H7N9-Bcc to provide evidence for the enhanced virulence of COVID-19-Bcc.

Particularly virulent and transmissible Bcc can cause outbreaks of clinical infections.[32] In this study, we detected several virulence-related, capsular polysaccharide-related, N-acyl homoserine lactone-dependent-related, Type IV pili (Tfp)-related, chemotaxis-related, and flagella-related genes. Bacterial fimbriae (pili) expressed on the cell surface are involved in the pathogenesis, mainly through adherence to host cells. In BCC, the pili gene associated with the common strain is cable (Cbl),[33] but the one detected in this study were pilA, pilB, and pilD. pilA, pilB and pilD as the key virulence factors in Tfp have been extensively studied in various Gram-negative pathogens.[34] Bacterial flagellin is the

only known ligand recognized by Toll-like receptor 5 (TLR5), which can cause increased inflammation and lead to lung damage.[35,36] The capsular polysaccharide is an important virulence factor because bacteria evade or counteract host immune responses through capsular polysaccharides.[16] It is worth noting that we have used statistical analysis to find that the virulence difference between COVID-19-Bcc and H7N9-Bcc may be related to the Chemotaxis CheA gene ($P<0.05$). Chemotaxis and its mechanism of signal transduction and response regulation have been well studied in Escherichia coli.[37] When the environment stimulates cell membrane-related receptors, they trigger autophosphorylation of the cytoplasmic histidine autokinase CheA, which forms a complex with the receptor through a coupling protein called CheW. CheA transfers its phosphate group to CheY can interact with the flagellar motor to switch its direction of rotation.[38] Studies have shown that chemotaxis and its mechanism of signal transduction and response regulation are necessary for bacteria to be fully virulent.[39] During growth in human serum, genes predicting the proteins required to develop bacterial chemotaxis and motility were up-regulated, indicating that chemotaxis and motility may be important during bacteremia.[40]

To investigate the evolutionary history of 49 Bcc and identify potential ancestral hosts and geographical reservoirs, kSNP3 was used to perform phylogenetic reconstruction. This study selected all *B. multivorans* and *B. cepacia* strains from the NCBI database from 2000 to the present for phylogenetic analysis. Phylogenetic analysis of the core genome revealed 177 *B. multivorans* and 92 *B. cepacia*. Analysis of *B. multivorans* showed that the source of the strains was mainly the clinical samples. As shown in Figure 6, ST1088 *B. multivorans* in this study was located in a co-clade with ST1325, and GCA_001529925 from the USA was in the same subclade as ST1088 *B. multivorans* in this study. GCA_001529925 was isolated from environmental samples in the USA in 2011. From the results of phylogenetic analysis, we can infer the possible evolutionary origin of ST1088 *B. multivorans* in this study. The geographical origins of ST1088 *B. multivorans* and ST1088 GCA_001529925 are distant, so they may have evolved independently. Analysis of *B. cepacia* revealed 50 isolates from clinical samples, accounting for 88.1%, including 28 strains of *B. cepacia* in this study. Except for strains with no exact ST type, the most popular ST type worldwide was ST9 (21.7%), followed by ST1578 (16.3%). The isolates closely related to *B. cepacia* in this study were the reference strains from China. Reference strains were obtained from Jiujiang City, China, and isolated from lake water. Although *B. cepacia* in this study was isolated in different years, all isolates showed a high degree of homology compared to the NCBI strains. The results of phylogenetic analysis indicated that COVID-19-Bcc may have evolved from H7N9-Bcc. However, there are fewer related studies on *Burkholderia* than ESKAPE. It is impossible to conduct a specific analysis of its origin and pathogenicity, suggesting that we should strengthen the detection and research on this type of bacterium.

Although the phenotype of highly virulent and multidrug-resistant COVID-19-Bcc strains is relatively well defined, the mechanisms behind the phenomenon are not clear. We hypothesise that there may be two reasons for this: firstly, neo-coronavirus infections may lead to impaired functioning of the host immune system,[41,42] and this immunosuppression may allow for a change in the host's internal environment, which may make it easier for the highly virulent and resistant strains to colonise; secondly, many neo-coronavirus-infected individuals will have a comorbidity of severe bacterial infections, and the widespread use of antibiotics may have been a major factor in the evolution of the strains towards hypervirulence and multidrug resistance.[43] In addition, highly virulent and drug-resistant strains may spread in hospitals through environmental contamination, medical equipment, and horizontal transfer of drug-resistant genes, posing a serious threat to public health. Therefore, how to address this challenge is an important issue.

While the development of new antibiotics against new bacterial targets is important, the rational use of antimicrobials is even more critical.[44,45] This includes strictly limiting the use of antibiotics, avoiding unnecessary antibiotic prescriptions, and ensuring that the correct type and dose of antibiotic can be given when it is really needed.[46] Hospitals should implement stringent infection control measures, including hand hygiene, isolation of infected patients, regular disinfection of medical equipment and the environment, and monitoring the development of antibiotic resistance. These combined measures can be effective in slowing the spread of resistance, protecting the effectiveness of antibiotics and providing a defence against possible future resistant strains.

Conclusion

By comparing 49 Bcc strains from patients co-infected with COVID-19 and H7N9, it was found that COVID-19-Bcc may have evolved from H7N9-Bcc, and that COVID-19-Bcc strains are multi-drug-resistant and more virulent compared to H7N9-Bcc strains, suggesting that we should strengthen the detection of and research on this group of bacteria.

Ethical Statement

This study was conducted according to the Declaration of Helsinki, and approval was obtained from the Institutional Review Board (IRB) at The First Affiliated Hospital of Zhejiang University. The ethics permit number is 2021IIT372. Due to the retrospective nature of the study and the anonymization of patient data, a waiver of informed consent was granted by the IRB. All patient data were handled with strict confidentiality and care to ensure privacy.

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Disclosure

The authors report no conflicts of interest in this work.

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