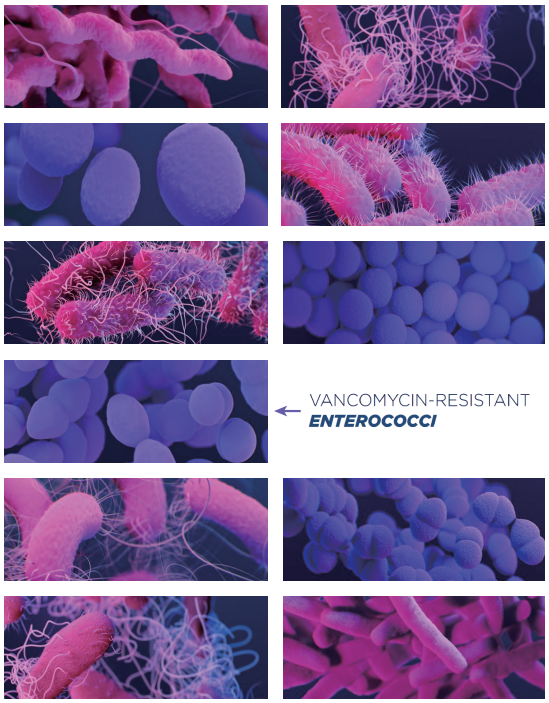
ISSN 2096-7071
CN 10-1629/R1

CHINA CDC WEEKLY

Vol. 4 No. 2 Jan. 14, 2022

CCDC weekly

中国疾病预防控制中心周报



ISSN 2096-7071
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China CDC Weekly

Cover Photos : Adapted from US CDC document (http://dx.doi.org/10.15620/cdc:8253).

Review

Antimicrobial-Resistant Evolution and Global Spread of *Enterococcus faecium* Clonal Complex (CC) 17: Progressive Change from Gut Colonization to Hospital-Adapted Pathogen

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ABSTRACT

For a long time, *Enterococcus faecium* (*E. faecium*) was thought to be a commensal strain in human and animal digestive tracts. However, over the past three decades, some unique *E. faecium* clones rapidly acquired multiple antimicrobial resistance (AMR), which led these clones to survive hospital environments and become a hospital-adapted *E. faecium* clonal complex (CC) 17. Since the adaptation of these clones to changes in habitat, vancomycin-resistant *E. faecium* CC17 has emerged as the leading cause of hospital-acquired infections worldwide. This epidemic hospital-adapted lineage has diverged from other populations approximately 75 years ago. The CC17 lineage originated from animal strains, but not human commensal lines. We reviewed the evolutionary progress and the molecular mechanisms of *E. faecium* CC17 from a gut commensal to a multi-antimicrobial resistant nosocomial pathogen.

INTRODUCTION

Enterococcus faecium (*E. faecium*) is becoming one of the leading causes of hospital-acquired infections. However, in the past few decades, *E. faecium* was thought to be a commensal of the animal digestive tract, or even a probiotic, and an important cause of nosocomial infections. The hospital-adapted lineage of *E. faecium* clonal complex (CC) 17 has caused enormous burdens for hospitals, which can cause severe morbidity and mortality (*1*). The resistance to ampicillin, vancomycin, and other microbials has made this lineage difficult to treat in hospital settings.

E. faecium is mainly classified into two types: one is community/commensal-associated *E. faecium* CC94, whereas the other is hospital-adapted *E. faecium* CC17 (*2*). By average nucleotide identity analysis (ANI), *E. faecium* CC17 and CC94 have nucleotide differences totaling more than 5%, and thus, above the threshold used for species identification (*3*). Compared with *E. faecium* CC94, the CC17 lineage contains pathogenicity islands (PAI) and plasmids or other mobile genetic elements (MGEs) associated with antimicrobial resistance (AMR), virulence, and/or colonization (*4*). This pathogenic lineage likely emerged from animal strains approximately 80 years ago, as a result of using antimicrobials to treat humans and animals. As such, an understanding of the progressive changes and the molecular mechanisms of this rapidly evolving lineage is of substantial interest and the first step in prevention.

OCCURRENCE OF *E. FAECIUM* CC17

The evolutionary trajectories of *E. faecium* CC17 and CC94 were different. Two main divergence events have occurred in the evolutionary path of *E. faecium* and the creation of *E. faecium* CC17. The first divergence event was estimated to have occurred approximately 2,776±818 years ago, concomitant with the increased urbanization and domestication of animals. During this bifurcation, *E. faecium* species were classified into human and animal dominant lineages. The animal lineage was further categorized into an epidemic hospital lineage (*E. faecium* CC17) and a lineage that colonized and spread in communities only, causing sporadic infections in animals and humans (*E. faecium* CC94). The second divergence event was thought to have occurred because of the invention and use of antimicrobials in medicine and agriculture approximately 74±30 years ago (*5*). These findings illustrated that the formation of *E. faecium* CC17 occurred in parallel with human behavior changes.

Another model using a synonymous single

nucleotide polymorphism (sSNP) molecular clock and estimations of *Escherichia coli* and *Bacillus anthracis* parameters predicted that the evolutionary division of *E. faecium* CC17 and CC94 occurred 1,000,000 to 3,000,000 years ago. Core genome analysis showed that the differences between the two subpopulations occurred at the core genomic level and long preceded the modern antibiotic era, or even long preceded the inhabitation of humans on earth (*6*). Admixture analysis showed a scarce number of recombination events between *E. faecium* CC17 and CC94.

A recent analysis of a global representative vancomycin-resistant *Enterococcus* (VRE) genome set estimated that the overall phylogenomic structure of vancomycin-resistant *E. faecium* CC17 was highly dependent on recombination (54% of the genome). The split between *E. faecium* CC17 and CC94 was estimated to have occurred more than 2,765 years ago. Molecular clock calculations suggested that the branching of animal isolates and clinical lineages occurred approximately 502 years ago (*7*). However, the discrepancy is difficult to resolve using existing methods, since accurate phylogenomic analyses rely on the assumptions that recombination occurs across a restricted region of the genome and that these regions can be reliably detected and removed. *E. faecium* CC17 genomic analysis revealed recombination across the whole genome, even in relatively small sample subsets. Consequently, molecular clock approaches are prone to inaccuracies, thereby providing an explanation for wide discrepancies in estimates (*8*).

SURVIVAL AND ADAPTATION IN HOSPITAL ENVIRONMENTS

E. faecium CC17 is a typical example of a cumulative evolutionary process that improved the relative fitness of bacteria in hospital environments. The successful survival and spread of this lineage in hospital environments favors rapid adaptation to more antimicrobials, especially first-line clinical antimicrobials. The high genome plasticity of *E. faecium* CC17 is one of the key characteristics that may explain why it successfully adapted to harsh conditions, such as hospital environments, and how it managed antibiotic and antiseptic stresses. Recombination was found to have a significant impact on the *E. faecium* CC17 genome and the acquisition of antimicrobial resistance genes. Interestingly, *E. faecium* CC94 established an important reservoir for donating foreign DNA to *E. faecium* CC17, and multiple recombinant regions comprise up to 26% of the *E. faecium* CC17 genome (*9*). The lack of clustered regularly interspaced short palindromic repeats-associated (CRISPR-*cas*) loci has also contributed to the adaptation of *E. faecium* CC17 in hospital environments. High recombination rates were commonly detected in vancomycin-resistant variants of hospital-adapted CC17 (*10*). Furthermore, MGEs play a crucial role in the environmental and nosocomial epidemic adaptation of hospital lineages. For example, vancomycin-resistant determinant *van* operons are always carried on the transposable element Tn*1549*, which accounts for the appearance and spread of *E. faecium* CC17 in hospital settings (*11*).

Phylogenetic and eBURST analyses of hospital-adapted *E. faecium* CC17 confirmed the existence of 3 separate hospital sub-lineages, originating from sequence types (STs) 17, 18, and 78 (*12*). The isolates originating from ST17 and ST18 contained a relatively high proportion of genomic text of pig isolates, while the ST78 lineage co-clustered with poultry-originating isolates (*12*).

E. faecium CC17 has several important clinical features, such as ampicillin resistance, vancomycin resistance, and the presence of the *esp* virulence factor, with the latter accounting for biofilm formation, urinary tract infections, and endocarditis. Once the *E. faecium* CC17 isolates acquired high invasive potential through horizontal gene transfer and adapted to a distinct pathogenic niche, the population was isolated and declined recombination with other populations (*12*). This corresponded with surveillant results, which indicated that hospital isolates commonly carried some resistance and virulence genes that were not detected in community/animal isolates (*E. faecium* CC94) (*9*).

AMR OF *E. FAECIUM* CC17

The worldwide ratio of *E. faecalis*-to-*E. faecium* infections in clinical settings has changed dramatically in favor of *E. faecium* CC17 after acquiring high resistance to multiple antimicrobials. In addition, the latter species is naturally resistant to cephalosporins and aminoglycosides at low levels, and the CC17 lineage is nearly always resistant to ampicillin (*13*). More importantly, vancomycin-resistant *E. faecium* CC17 has spread globally in the past few decades (*14*). As such, a better understanding of the resistance mechanisms of this pathogen is needed for the prediction and prevention of its dissemination.

Resistance to ampicillin is a primary trait of *E. faecium* CC17. In the United States, nosocomial infections caused by ampicillin-resistant *E. faecium* CC17 increased in the 1980s, followed by the emergence of vancomycin-resistant *E. faecium* CC17 in the 1990s. In Europe, vancomycin-resistant *E. faecium* CC17 prevalence rates have been increasing since the 2000s. These findings strongly suggest that the emergence and spread of ampicillin-resistant *E. faecium* CC17 in hospitals has preceded the dramatic emergence of vancomycin-resistant *E. faecium* CC17. Hence, efforts for preventing the further spread of this epidemical pathogen should focus on the early disclosure of ampicillin-resistant *E. faecium* CC17 strains.

Ampicillin resistance in *E. faecium* CC17 is due to 1) alterations caused by mutations in penicillin-binding protein (PBP5), resulting in lower affinity; and 2) overproduction of PBP5 (*5*). *E. faecium* isolates of hospitals acquired selective advantage after obtaining ampicillin resistance and some virulence genes. After successfully exploiting the hospital environment, the adaptive isolates increased in frequency to become the dominant clones. By the "genetic capitalism" strategy, the dominant isolates acquired additional adaptive mechanisms more easily, such as vancomycin resistance, thereby fully adapting as a nosocomial pathogen that spread globally (*15*). Several studies have reported that the nucleotide difference of the PBP5 gene between ampicillin-resistant and -sensitive *E. faecium* isolates was 5%. The mutation of the PBP5 gene may be the reason for the ampicillin resistance phenotype (*6*).

The *van* genes, especially *vanA*, *vanB*, and *vanM*, carry greater clinical significance, as they can confer intermediate-to-high levels of resistance to vancomycin and are encoded on MGEs. In some European countries, 30% to 50% of *E. faecium* CC17 isolates showed vancomycin resistance, and this was considered the greatest threat to successful clinical treatment. In China, the prevalence of vancomycin-resistant *E. faecium* (VREfm) has been considered as low as 3.6% according to the report from 2010 China Antimicrobial Surveillance Network (CHINET). However, a monitoring data covering 45 tertiary hospitals indicated that the incidence of VREfm had increased to 14.3% in 2013 (*16*). Due to the conjugative transposons and plasmids, the dissemination of vancomycin resistance was expanded among enterococcal strains, species, and even genera such as *Staphylococcus aureus*. Consequently, VREfm are already the second most common nosocomial pathogen in the United States after heavy use of vancomycin in clinical settings (*4*).

Similarly in Europe, VREfm colonization and infection dramatically increased over a short period of time. However, unlike in the United States, VREfm colonization was limited in hospitals, and large community spreading was thought to be one reason for the sudden increase in VREfm colonization and infection. In the late 1980s, farmers in Europe began adding to animal feed avoparcin, a glycopeptide antimicrobial-like vancomycin. After this, VREfm colonization was soon observed in farm animals as well as in the community. The use of avoparcin in the animal industry was subsequently banned in Europe in 1996. However, persistent VREfm colonization in poultry has been reported up to eight years after the ban (*17*).

The optimal therapy for VRE infections in clinical settings remains uncertain. The new antibiotics daptomycin and linezolid are the most utilized last-line antibiotics. However, mutations in any one of the three genes, *liaF*, *liaS*, and *liaR*, have been linked to daptomycin resistance, while mutations in 23S rRNA, the Cfr rRNA methyltransferase gene, or *optrA* have been reported to cause linezolid resistance. Although plasmid-mediated linezolid resistance can lead to sporadic outbreaks, resistance to last-line antibiotics remains uncommon (*8*).

Transferable resistance poses a great threat, as it can produce a much greater threat due to its wide and rapid dissemination. Several reports have suggested that the acquisition of insertion sequence (IS) elements can facilitate the niche adaptation of *E. faecium* CC17 by increasing its genome plasticity. These findings indicate that the global emergence of *E. faecium* CC17, as observed since 1990, represents the evolution of the CC17 lineage with better adaptation (AMR) than other *E. faecium* lineages to the constraints of hospital environments (*10*).

COLONIZATION AND VIRULENCE OF *E. FAECIUM* CC17

E. faecium CC17 can infect or persistently colonize human hosts depending on progressively acquired genetic elements that confer selective advantages. These acquired genetic elements include the antimicrobial resistant genes (ARGs) and virulence genes. In addition to the ARGs, the colonization and virulence genes are

also important for the adaptability and spread of *E. faecium* CC17 to hospital environments and/or patient niches. These colonization and/or virulence genes mainly include the *fms* genes encoding microbial surface components that recognize adhesive matrix molecules, esp_{Efm} genes encoding surface proteins responsible for biofilm formation, and hyl_{Efm} genes encoding putative glycoside hydrolases that facilitate intestinal colonization and peritoneal invasion (*18*). These virulence genes are often co-localized in putative pathogenicity islands (PAIs) or mobile elements, thereby facilitating their spread between isolates. PAIs are large elements that can be acquired by horizontal transfer and confer virulence to bacterial pathogens. The VREfm of 45 tertiary hospitals monitored in 2013 contained the esp_{Efm} gene with the frequencies of 89.9% (62/69), while 27.5% (19/69) of the VREfm strains carried hyl_{Efm} gene (*16*). The third-generation cephalosporins resistant *E. faecium* CC17 increased the risk of colonization and infection in hospitals. In hospitals, *E. faecium* was found to remain viable on inanimate surfaces from 7 days to 2 months, which increases the risk of acquiring ARGs and virulence genes (*1*).

SUMMARY

Multiple AMR *E. faecium* CC17 outbreaks have not only incurred significant costs for healthcare systems but also placed vulnerable patients at higher risk of acquiring fatal infections. The patterns of *E. faecium* CC17 variation illustrate that new phenotypes are likely to continue to emerge, driven by local variations in selective stress and access to distinct gene pools via both homologous recombination and an extensively mobilizable pangenome. These genomic features suggest that controlling the hospital spread of *E. faecium* CC17 will remain challenging. The successful control of AMR *E. faecium* CC17 outbreaks often mentions the importance of general infection control procedures, such as education for healthcare workers, sanitation of hands and environments, antimicrobial stewardship, and use of sterile equipment and personal protective gear. The use of molecular typing, rapid *van* gene detection, and AMR surveillance can help to identify outbreaks early, allowing infection control to limit the spread of the outbreak. Antimicrobial stewardship practices can limit the dissemination of antimicrobial resistance genes in *E. faecium* CC17, extending the efficacy of current antimicrobials.

Conflicts of interest: No conflicts of interest.

Funding: Supported by Chinese Academy of Medical Science (CAMS) Innovation Fund for Medical Science (CIFMS 2019-12M-5-024) and National Natural Science Foundation of China (32172314).

doi: 10.46234/ccdcw2021.277

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Submitted: December 01, 2021; Accepted: December 21, 2021

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Perspectives

Public Health Control Measures for the Co-circulation of Influenza and SARS-CoV-2 During Influenza Seasons

John S Tam[1,2,#]; Yuelong Shu[2,3]

SEASONAL INFLUENZA IN THE MIDST OF COVID-19

The World Health Organization (WHO) named the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as coronavirus disease 2019 (COVID-19) and declared the outbreak a Public Health Emergency of International Concern (PHEIC) on January 30, 2020 and a pandemic on March 11, 2020. Globally, there have been 239,437,517 confirmed cases of COVID-19 reported to WHO, including 4,879,235 (2.1%) deaths as of October 15, 2021 (*1*). The COVID-19 pandemic continues to cause an unparalleled impact on global public health security and economic well-being in the context of previous influenza pandemics as well as other emerging infectious diseases in history (*2*). As the epidemiology, clinical presentations, and control measures for SARS-CoV-2 and influenza share many features, there is a need to develop strategies to address additional challenges arising in the continued surveillance, prevention, and clinical management of influenza in conjunction with COVID-19 pandemic responses. SARS-CoV-2 and influenza are expected to be circulating during the upcoming influenza season and may lead to situation where an amplified respiratory disease burden occurs due to both viruses spreading, causing overlapping symptoms or severe clinical illness particularly in the case of co-infections (*3*). Therefore, it is essential that effective public health control measures are in place for the forthcoming influenza season to protect those at risk (e.g., the elderly and patients with underlying chronic diseases), prevent severe illness, and minimize additional impact on the healthcare system and a surge in hospital admissions.

EPIDEMIOLOGY OF INFLUENZA VIRUSES DURING COVID-19 PANDEMIC

To understand the epidemiology of influenza with an ongoing circulation of SARS-CoV-2, it is essential to understand the general transmission profiles of both competing viruses. The median basic reproduction number (R_0) of seasonal influenza was estimated to be 1.28 (*4*). The estimated R_0 of the initial strain of SARS-CoV-2 was reported to be 2.79 (*5*), which explained the enhanced transmission of SARS-CoV-2 as observed during the early phase of the pandemic as compared to the transmission of seasonal influenza. The Alpha (B.1.1.7) variant of SARS-CoV-2 emerged in the United Kingdom and was the first variants of concern (VOC) to show enhanced transmissibility (43% to 90% over the ancestral strain in UK) as well as subsequent VOCs (Beta — 50% in South Africa; Gamma — 1.7% to 2.4% in Brazil) (*6*–*7*). The Delta (B.1.617.2) variant was first detected in India and showed an estimated R_0 of 5.08 (*8*) and an enhanced transmission rates of 60% over that of the Alpha VOC (*6*). The Delta variant has replaced the other VOCs, invigorating repeated outbreaks in countries previously able to suppress COVID-19 circulation as well as resurgence of COVID-19 disease in countries with high vaccination coverage (*9*). The heightened transmissibility of SARS-CoV-2 will likely affect the spread of respiratory viruses and the epidemiology of influenza in the coming seasons. The differences in transmission profiles of SARS-CoV-2 and influenza may also reflect prior infection and vaccination in previous influenza seasons, conferring a level of population immunity against seasonal influenza, compared with the lack of population immunity to SARS-CoV-2.

Information from the United States (*10*) and several countries (*11*–*15*) in the Northern Hemisphere on seasonal influenza activities during the early 2020 showed sharp declines in the number of influenza infections for the traditional high winter season for influenza. In the Southern Hemisphere, Australia, Chile, South Africa, and New Zealand reported similar observations during their influenza season in 2020 (*10*,*16*–*17*). This phenomenon is further demonstrated using data collected on influenza surveillance in Hong

Kong, China (Northern Hemisphere) and Australia (Southern Hemisphere) over multiple influenza seasons to October 2021. As shown in Figure 1, laboratory confirmed influenza infections for Hong Kong, China and Australia were drastically reduced during influenza seasons for 2020 and 2021. Similar finding were reported from China (*18*).

In addition to influenza viruses, the etiology and epidemiology of traditional infections has also been significantly altered with notable decreases in the incidence of other seasonal respiratory viral infections. With the exception of rhinoviruses (RV), the incidence of respiratory syncytial virus (RSV), parainfluenza viruses (PIV), adenovirus (AV), human metapneumovirus (hMPV), and other seasonal coronaviruses were effectively absent during surveillance activities for respiratory viruses during the COVID-19 pandemic period as reported from Australia (*16*), UK (*15*) and Canada (*19*).

Many studies suggested that the decline in the activities of seasonal influenza and other respiratory viruses may have been attributed to the widespread and stringent community non-pharmaceutical intervention (NPI) measures implemented to control the COVID-19 pandemic (*10–11,16–17*). These included the following: 1) border closure and quarantine of travelers for the control of importation; 2) community control measures such as widespread testing, isolating cases, contact tracing, and quarantine of exposed persons; 3) physical distancing measures such as stay-at-home orders, cancelling business and social gatherings, and school closures; 4) good personal and environmental hygiene including mandatory face mask policies in public areas; and 5) campaigns on risk communication to public and community stakeholders. Many of such measures implemented for COVID-19 control were also suggested to be effective for the control of influenza (*18,20–21*). In addition to these NPI measures, promotion of influenza vaccination had also been implemented. The increase in influenza vaccine uptake for 2019/2020 and 2020/2021 season was suggested to be a major factor contributing to the reduced burden of influenza of the seasons in Hong Kong. Influenza vaccination statistics in Hong Kong demonstrated 38%, 26%, 24%, and 10% increases in vaccine uptake among children aged 6 months to <6 years, children aged 6 to <12 years, adults aged 50–64 years, and the elderly aged ≥65 years, respectively (*22*). Similar increase in influenza vaccination rates was also noted in UK for the 2020/2021 season (*15*).

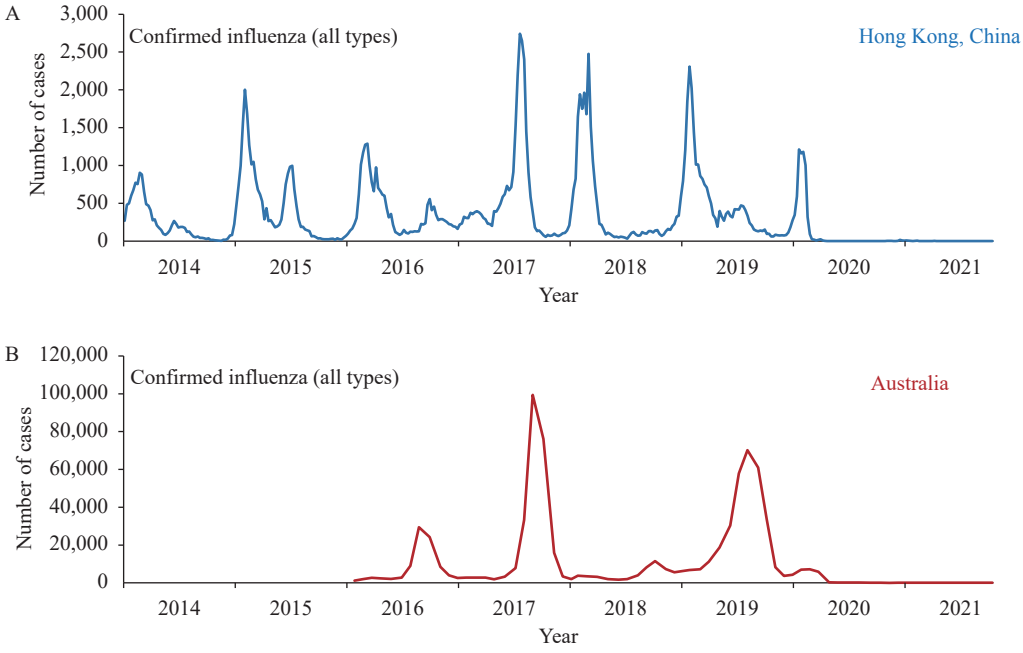

FIGURE 1. Laboratory-confirmed influenza infections in Hong Kong, China and Australia. (A) Influenza cases (January 2014–October 2021); (B) Influenza cases (January 2016–September 2021).
Notes: Figure 1A data source: Flu Express, Centre for Health Protection, Hong Kong Special Administration Region, https://www.chp.gov.hk/files/xls/flux_data.xlsx. Figure 1B data source: Immunisation Coalition, Australia, https://www.immunisationcoalition.org.au/news-data/influenza-statistics/.

INCIDENCE AND CLINICAL SIGNIFICANCE OF CO-INFECTION

With the ongoing intense surveillance of pathogens during the COVID-19 pandemic, the recovery of other pathogens in patients with SARS-CoV-2 infection has been reported (*23*–*25*). A recent systematic review and meta-analysis on the occurrence of co-infections and superinfections and their outcomes among patients with SARS-CoV-2 infection showed that the pooled prevalence of co-infection amounts to 19% and that of superinfection was 24% in 118 publications included in the systemic review (*25*). Among viruses identified in the analysis, influenza A had the highest prevalence (22.3%) followed by influenza B (3.8%) among co-infected patients while rhinovirus was the most frequent in patients with superinfection (11%). It is important to note from the analysis that patients identified with a co-infection or superinfection had higher odds of dying (odds ratio=3.31) than those who had SARS-CoV-2 infection alone. Patients with co-infections had a higher average length of hospital stay than those with superinfections (29.0 days *vs*. 16 days), and those with superinfections had a higher prevalence of requiring mechanical ventilation (45% *vs*. 10%) than those with a co-infection. Such information stimulated many discussions about the possible impact of the coming influenza season while variants of SARS-CoV-2 are circulating at the same time (*26*–*29*).

As discussed above, influenza infection may induce severe clinical disease due to superinfection or co-infection with SARS-CoV-2 (*24*). Bai et al. (*30*) in their research provide the first experimental evidence which may explain the mechanism by which co-infection of influenza virus and SARS-CoV-2 showed enhancement in pathogenesis. It was reported that co-infection was associated with an increased expression level of ACE2, the major receptor for SARS-CoV-2 entry into target cells, leading to the augmentation of SARS-CoV-2 infectivity. It was further observed in another study that simultaneous or sequential co-infection of SARS-CoV-2 and A (H1N1)pdm09 caused more severe disease as compared to single infections by either virus in hamsters (*31*).

CONTROL FOR CO-CIRCULATION OF SEASONAL INFLUENZA AND SARS-COV-2

The possible impact of influenza virus and SARS-CoV-2 co-circulating this autumn and winter season in the Northern Hemisphere has the potential to further impact the already strained public healthcare system under the COVID-19 pandemic, particularly on inpatient and intensive care utilization (*32*). Modeling studies on seasonal influenza implicated that as the number of seasons with low influenza activity increases, immunity in the population decreases with an increasingly susceptible population, leading to a possible 20% increase in influenza-related hospitalizations in the subsequent year if nonpharmaceutical intervention practices were eased (*29*, *33*). More broadly, it is suggested that healthcare systems should fully optimize available effective strategies for influenza management in anticipating future cocirculation of influenza and SARS-CoV-2 as the 2021–2022 influenza season approaches.

Diagnosis and Surveillance

The early symptoms of COVID-19 patients often include fever, dry cough, and fatigue, and it is not possible to distinguish SARS-CoV-2 infection from those of influenza based on symptoms alone (*34*–*35*). As best practices of care for the two infections are different, making available the rapid diagnostic tests for both viruses is essential, particularly for high-risk groups and patients with severe respiratory illnesses, in the situation that co-circulation of SARS-CoV-2 and influenza viruses is anticipated. In addition, rapid diagnostic testing and surveillance are necessary to ensure effective infection control procedures, including isolation, contact tracing, quarantine of exposed individuals, and containment measures in the community or institutional/hospital settings, can be implemented swiftly.

Vaccination

Influenza vaccination of risk groups as well as healthcare workers is central in seasonal influenza control measures. The WHO and other national and international health authorities had repeatedly made influenza vaccination recommendations. However, vaccine uptake among high-risk groups and healthcare workers remained low and vaccine uptake in the elderly population remained below the WHO recommended 70% coverage even in many high-income countries (*36*). Additional efforts to improve influenza vaccination rates among high-risk groups and healthcare workers are an essential and effective strategy to reduce influenza burden and allow for

better preparedness for anticipated co-circulation of influenza and SARS-CoV-2 in the coming influenza season (*37*). In addition, the ability of the influenza virus to augment COVID-19 severity (*31*) underscores the importance of influenza virus as a key target for prevention and control of severe clinical disease due to co-infection. Therefore, influenza vaccination should be recommended for populations with a high risk of co-infection.

Treatment

Influenza vaccines vary in degree of antigenic match to circulating viruses, and influenza vaccine effectiveness can differ by age group as well as the degree of antigenic match between vaccine and circulating viruses (*38*). A number of neuraminidase inhibitors (NAIs) such as oseltamivir as well as inhibitors targeting the viral polymerase such as baloxavir, had been evaluated and demonstrated to be effective for the prophylaxis and treatment of influenza (*39–40*). However, no antiviral has been approved for the treatment of SARS-CoV-2 infection to date. In anticipation of cocirculation of influenza and SARS-CoV-2, strategies on influenza antiviral use should be developed to provide high-risk individuals with antivirals prophylactically and patients are treated within the 48-hour window according to established treatment guidelines. Prophylactic use of influenza antivirals may also be warranted during influenza outbreaks in care homes or institutions as an additional protective measure to reduce influenza burden and burden of co-infection in the most vulnerable populations.

CONCLUSION

The emergence of SARS-CoV-2 has resulted in an unprecedented global pandemic causing substantial morbidity and mortality, particularly among older and vulnerable adult populations. Public health policymakers worldwide have instituted stringent non-pharmaceutical interventions to mitigate the transmission of SARS-CoV-2 virus. Vaccines for SARS-CoV-2 have been developed and global vaccination for high-risk populations has been implemented gradually. There was concern regarding the potential of increased healthcare burden from the dual impact of an ongoing COVID-19 pandemic coinciding with the seasonal influenza virus peak which may cause significant additional morbidity, mortality, and health-service demand. Experimental and surveillance reports also indicated that co-infection with influenza viruses and SARS-CoV-2 occurs with enhanced severity. As the 2021–2022 Northern-Hemisphere influenza season approaches, it is important to maintain a high index of suspicion for co-infection. Measures should be adopted to prevent co-infection. Vaccination against influenza becomes even more important. Rapid diagnostic evaluation of patients presenting in respiratory distress to emergency departments for both SARS-CoV-2 and influenza is necessary. Treatment with antiviral agents for influenza should be initiated. Moreover, social distancing and mask wearing are beneficial to protect people from the transmission of either or both viruses.

doi: 10.46234/ccdcw2021.228

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Submitted: October 25, 2021; Accepted: October 28, 2021

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Perspectives

What is the Potential Cause for the Predominance of GII.2[P16] Norovirus in Acute Gastroenteritis Outbreaks in China?

Jie-mei Yu[1,#]

ABSTRACT

GII.2[P16] noroviruses (NoV) reemerged and rapidly became the main epidemic strain in acute gastroenteritis (AGE) outbreaks in Asian countries since 2016. The current GII.2 [P16] NoV showed the same antigenicity to the ones before 2016, but several unique amino acid substitutions existed in the RNA dependent RNA polymerase (RdRp) and other non-structural proteins, and the viral load of the current GII.2[P16] NoV was higher than those of other genotypes, it was estimated that the viral replication ability may have improved. However, other genotypes, such as GII.1 and GII.3, also had recombination with the novel RdRp, were not prevalent in AGE-outbreaks; thus, it was inferred that the capsid proteins also played an important role in the enhanced replication process. The viral infection could also be affected by other factors, such as the population genetic background, the climate and environment, and people's lifestyles. Continued surveillance on genetic diversity and evolutionary pattern for the GII.2[P16] NoV is necessary.

INTRODUCTION

Human norovirus (NoV) is the leading cause of epidemics of viral acute gastroenteritis (AGE) worldwide, affecting people in all age groups. Data showed that NoVs were genetically diverse and played an increasingly important role in the etiology of AGE in China (*1*), among which GII.2[P16] recombinant NoV reemerged and caused outbreaks in some Asian countries like China and Japan in 2016. The mechanism behind the sudden epidemic is poorly characterized. In this study, we summarized and analyzed the major potential reasons for the re-emergence and the prevalence of GII.2[P16] NoV.

Genomic Feature and Genetic Diversity of NoV

NoVs are non-enveloped, single-stranded, positive sense, polyadenylated RNA viruses that belong to the *Norovirus* genus, Caliciviridae family. The genome is approximately 7.3–7.7 kb in length and consists of three open reading frames (ORFs): ORF1 encodes a polyprotein that is further cleaved into six nonstructural proteins (P48, NTPase, P22, VPg, Pro, and RdRp); ORF2 encodes the major capsid protein VP1 and can be divided into shell (S) and protruding (P) domains, and the P domain is further subdivided into P1 and P2 subdomains, where P2 is a hypervariable region, determining the antigenicity and cell binding of the virus; ORF3 encodes the minor structural protein VP2. The genomes begin with a 5' end terminal pGpU sequence that is covalently linked to VPg, and a short-conserved region (CR) at the 5' end is repeated internally in the genome near the beginning of a subgenomic-sized RNA transcript (Figure 1).

NoVs have high genetic and antigenic diversity. Based on amino acid diversity of the complete VP1 and nucleotide diversity of the RdRp, NoVs can be divided into 10 (GI–GX) genogroups with 48 confirmed genotypes and 60 confirmed P-types (*2*). GII genogroup viruses are the most commonly detected in humans; they can be further divided into 27 confirmed genotypes and 37 P types. From the mid-1990s to 2014, GII.4 genotype and its new variants have caused about 70%–80% of all NoV-associated AGE outbreaks worldwide (*3*). However, non-GII.4 NoVs have also severely affected China and some other Asian countries; in 2014 to 2015, GII.17 NoV emerged and increased in prevalence, while since 2016, GII.2[P16] reemerged and became predominant in AGE outbreaks.

Potential Factor for the Reemergence of GII.2[P16] in China

Though the GII.2 genotype has been reported since the 1970s, it was only detected in sporadic cases and accounted for less than 2% of all NoV genotypes. GII.2[P16] recombinant NoV first appeared in Japan

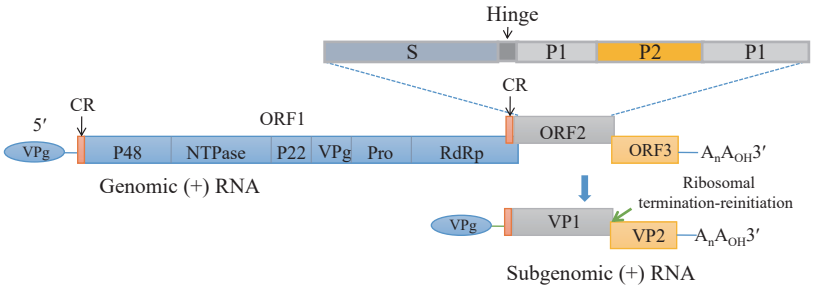

FIGURE 1. NoV genome structure and the encoding proteins.
Note: ORF1 encodes a polyprotein further cleaved into six nonstructural proteins. ORF2 encodes the major capsid protein and can be divided into S and P domains — the P domain is further subdivided into P1 and P2 subdomains. ORF3 encodes the minor structural protein.
Abbreviations: CR=conserved region; ORF=open reading frame; RdRp=RNA dependent RNA polymerase; S=shell domain; P=protruding domain.

in 2008 and caused AGE outbreaks in Osaka at that time and then was occasionally detected in sporadic cases. In 2016, GII.2[P16] NoV reemerged in Guangdong Province, China, and rapidly became the main epidemic strain in Asian countries (*4*–*6*). The occurrence of GII.2[P16] from sporadic to large-scale outbreaks suggested that the reemergence and sudden epidemic may be related to the change of viral biological properties, which led to stronger transmissive and infective ability.

Multiple amino acid changes at antigenic sites have always been the most important factors for the emergence of an antigenically distinct GII.4 virus (*7*). However, compared with the GII.2 viruses from before 2016, the currently prevalent GII.2[P16] has no unique amino acid changes on VP1, and phylogenetic analyses revealed that capsid protein did not play a role in the potential of GII.2 NoV to become an epidemic (*8*). It was also shown that different GII.2 strains circulating between 1976 and 2010 had undergone a limited amount of evolution in blockade epitopes (*9*). All these results revealed that the reemergence of GII.2[P16] NoV was not caused by the antigenic drift of capsid proteins. On the contrary, compared with GII.P16 RdRp detected before 2016, five unique amino acid substitutions (D173E, S293T, V332I, K357Q, and T360A) were found in the novel GII.P16 RdRp; except for V332I, all were located on the surface of the protein. Since a single amino acid change on the RdRp surface of GII.4 NoV can affect the biological function (*10*), it is speculated that the unique amino acid changes on RdRp of GII.2[P16] could have certain impacts on the viral replication activity. Indeed, it was observed that the viral load of current prevalent GII.2[P16] NoV was higher than those of GII.4 and GII.17 genotypes in the whole population (*11*), suggesting that the enhanced replication ability was an important factor for the recurrence of GII.2[P16] NoV.

RdRp is a key protein that determines the replication efficiency of the NoV genome, and amino acid substitutions on it may alter the replication kinetics or fidelity of the virus. However, besides GII.2, the emerging novel GII.P16 RdRp has been recombined with seven other different capsid genotypes: GII.1, GII.3, GII.4, GII.12, GII.13, GII.16, and GII.17. A total of 312 nearly full genome sequences of these different genotypes were downloaded from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank) in this study. Amino acid comparison results showed that, except for GII.13, GII.16 and GII.17 still recombining with GII.P16 RdRp before 2016, the capsids from the 5 other genotypes after 2016 were recombined with the novel GII.P16 RdRp (Table 1). However, among these 5 different novel recombinant genotypes, GII.2[P16] caused 81.2% of the NoV-related AGE outbreaks in China (*12*), indicating that the novel GII.P16 RdRp was not the only factor that enhanced the viral replication ability. As a matter of fact, previous studies have determined that NoV proteins P48, VP1, and VP2 can modulate GII.4 RdRp activity in a species-specific manner (*13*). Compared with the GII.2[P16] before 2016, excluding RdRp, the current GII.2[P16] had 9 unique amino acid sustitutions in other nonstructural proteins. Amino acids in position 78-79 in P48, 147 in P22, and 49 in Pro of the viruses that had the novel GII.P16 RdRp were all EE, Q, and I. Except GII.12, the amino acids in position 165 of P48, 312 of NTPase, and 52 of P22 on the viruses that had novel GII.P16 RdRp were all R, P, and R, respectively (Table 1). This phenomenon suggested that the P48,

TABLE 1. Amino acid comparisons of nonstructural proteins between different NoV genotypes/variants recombined with GII.[P16] RdRp.

Genotypes/Variants	P48			NTPase	P22			Pro	RdRp				
	52	78-79	165	312	52	147	158	49	173	293	332	357	360
GII.2 2009−2014	N	−	K	A	K	P	V	V	D	S	V	K	T
GII.2 2010−2012	N	E-	K	S	K	P	A	V	D	S	V	K	T
GII.2 2016−2019*	K†	EE§	R†	P†	R†	Q§	T†	I§	E§	T§	I§	Q§	A§
GII.4 2013	NA	NA	K	P	R	S	V	V	D	S	V	K	T
GII.4 2016−2019*	E/G†	EE§	R†	P/S†	R†	Q§	A/T†	V/I	E§	T§	V/I	Q§	A§
GII.3 2011−2015	N	E-	K	P/S	K	P	A	V	D	S	V	K	T
GII.3 2016−2018*	E†	EE§	R†	P/S†	R†	Q§	T†	I§	E§	T§	I§	Q§	A§
GII.1 2016−2018*	K/E†	EE§	K/R†	P†	R†	Q§	P/S¶	I§	E§	T§	I§	Q§	A§
GII.12 2017−2018*	K†	EE§	K¶	S†	K¶	Q§	P¶	I§	E§	T§	I§	Q§	A§
GII.13 2011−2015	N	E-	K	S	K	P	A/T	V	D	S	V	K	T
GII.13 2016−2018*	N/K	E-/EE	K/R	P/S	K/R	P/Q	A/T	V/I	D/E	S/T	V/I	K/Q	T
GII.16 2018*	N	−	K	A	K	P	A	V	D	S	V	K	T
GII.16 2012	N	E-	K	S	K	S	A	V	D	S	V	K	T
GII.17 2014	N	E-	K	A	K	P	T	V	D	S	V	K	T

Note: "−" stands for amino acid deletion; "/" stands for "or," e.g., R/K stands for R or K in the site.
Abbreviations: RdRp=RNA dependent RNA polymerase; NoV=norovirus; NA=not applicable.
* The GII.P16 NoVs currently prevalent.
† Key amino acid sites with changes on the currently circulating NoVs that had the novel GII.P16 RdRp.
§ Unique amino acids in currently predominant viruses.
¶ Different amino acids between currently circulating GII.[P16] strains.

NTPase, P22, and Pro together with the novel GII.P16 RdRp may enhance the viral replication ability and the fitness to the host.

As described above, VP1 can enhance the RdRp activity of NoV, we inferred that GII.P16 RdRp recombined with different types of capsid may potentially have different effects on the replication ability of the recombinant viruses, which needs further detailed scientific support. Furthermore, compared with the rare prevalent genotypes, the long-term prevalent ones like GII.4 would have a better fitness and stronger transmission ability in the population, and indeed, GII.4 [P16] was the primary cause of NoV outbreaks in the United States, Canada, and many other non-Asian countries at present (*14–15*). The regional specificity of GII.2[P16] and GII.4[P16] has little relation to the biological properties of the virus itself, while it may be attributed to the differences in the population's genetic background and lifestyes, the climate, the environment, and so on.

Potential Evolutionary Pattern of GII.2[P16] NoV in the Future

A previous study suggested that the non-GII.4 genotype NoVs were static with their capsid as they were unable to evolve antigenically, and they would be prevalent for only a short period in limited areas and populations and then would shift to another genotype (*8*). Until now, GII.2[P16] NoV has been the predominantly circulating strain in China and in many Asian countries for more than five years since its reemergence in 2016. The evolutionary patterns for the viruses are unclear. Will the virus disappear after a period of epidemic? Or will the virus evolve into new variants that can lead to new outbreaks by adapting to new susceptible populations and drive escape from herd immunity every 2–7 years just like GII.4 did? Or will the virus generate new receptor-binding sites like GII.13 and GII.21 did? Or will the virus optimize receptor-binding sites for enhancing receptor-binding ability just like GII.17 did? Or even, will the virus acquire another novel RdRp and produce novel recombinant just like it already did? So, it is important to trace the genomic signatures and dynamic evolution of the GII.2[P16] NoV, which can provide scientific evidence and guidance for the prevention and control of the virus.

Funding: Fundamental Research Funds for the Central Universities (2020RC016).

doi: 10.46234/ccdcw2022.003

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Submitted: September 18, 2021; Accepted: October 29, 2021

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Notes from the Field

Cholera Caused by a New Clone of Serogroup O1 *Vibrio cholerae* — Beijing Municipality, China, June 2021

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Several lineages have been identified in the population of serogroup O1 *Vibrio cholerae* (*V. cholerae*) (*1–3*). The strains, which were responsible for the ongoing seventh cholera pandemic, were in Lineage 2. Nearly all the *V. cholerae* strains in this lineage carried genes coding cholera toxin (*ctxAB*) (*1–2*). Lineage 3b consists of strains isolated from different continents and the vast majority of strains in this lineage lack the *ctxAB* genes.

In China, toxigenic serogroup O1 *V. cholerae* strains were rarely isolated after 2010 (*4*). However, 2 serogroup of O1 *V. cholerae* that possessed the *ctxAB* genes were isolated from patients in June 2021. The first and second case were found after visiting doctors in the same hospital in Beijing on June 19 and 21, 2021, respectively. Both patients complained of abdominal pain and watery diarrhea about 5 times a day, which started on June 17 and 18, respectively. No erythrocytes and leukocytes were observed by stool microscopy. There was no epidemiological connection between these two cases. Neither patient had a common history of exposure or had a common travel history. The first patient stayed in Beijing and the second one traveled to Tianjin 3 days before onset of symptoms. No contacts of the two patients complained of having diarrhea symptoms. Both patients recovered after receiving antibiotic therapy. Real-time polymerase chain reaction (PCR) assay showed that the fecal specimens were positive for the *ctxAB* and *rfb* gene of O1 *V. cholerae* (*5*). *V. cholerae* strains (named BJVC202101 and BJVC202102) were obtained on June 20 and 22 from the specimens of the first and second cases, respectively, and both were serogroup O1, serotype Ogawa.

Genome sequencing analysis indicated that both of these 2 genomes harbored 2 tandem copies of CTX prophage (*6*), which both carried genes for $rstR^{class}$ and $ctxB^{10}$ (Figure 1C). Vibrio pathogenicity island 1 (VPI-1) and VPI-2 were detected but Vibrio seventh pandemic island 1 and 2 were absent in them. These 2 genome sequences were combined with a representative global serogroup O1 *V. cholerae* genome collection (*1–3,7*) for phylogenetic analysis using a pipeline in the China Pathogen Identification Net (China PIN). These 2 strains formed a cluster, and it was most closely related to the *V. cholerae* isolated in Ukraine and Russia (*8*) in the phylogenetic tree based on the non-repetitive, non-recombinant core genome single nucleotide polymorphisms (SNPs) (Figure 1A). We reconstructed a phylogenetic tree based on the non-repetitive, non-recombinant core genome SNPs of 18 (including these 2 genomes) most closely related genomes (Figure 1B). The SNP number between these 2 isolates was 8, and varied from 894 to 2,231 between this cluster and the Ukraine and Russia isolates. Therefore, these 2 strains were genetically closely related but remotely related to the Ukraine and Russia ones.

In conclusion, these 2 strains were most closely related to the genomes in Lineage 3b. We prefer to take these isolates as a new clone. The existence of *ctxAB* indicated the potential of this clone to cause cholera outbreaks. Moreover, no epidemiological connection between the 2 patients was observed, which may indicate the wide distribution of this clone of *V. cholerae*. Therefore, surveillance on this clone should be carried out.

Acknowledgments: Staff of Intestinal Department of Outpatient, Aviation General Hospital of China Medical University.

doi: 10.46234/ccdcw2021.279

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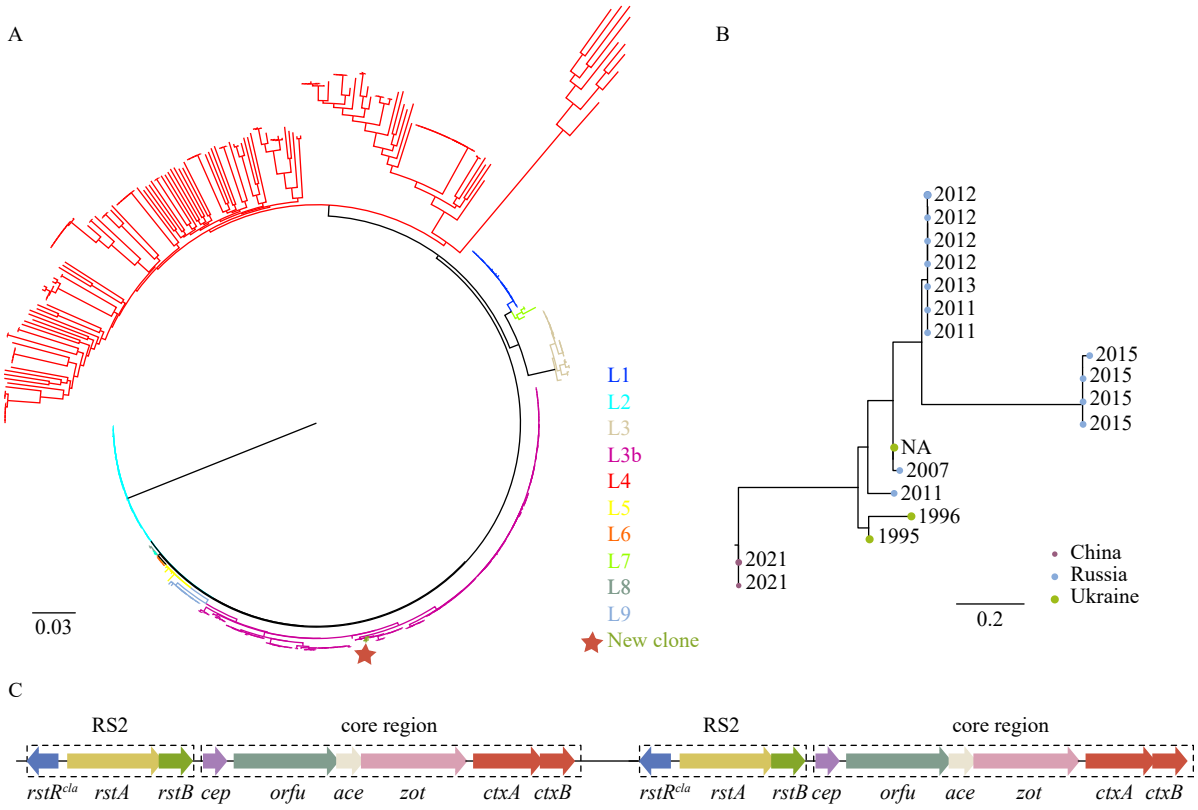

FIGURE 1. Phylogenetic analysis and CTX prophage structure of these two *V. cholerae*. (A) Maximum-likelihood tree constructed for SNPs identified in the non-repetitive, non-recombinant core-genome of the genome collection. (B) The maximum-likelihood tree was constructed on the SNPs identified in the non-repetitive, non-recombinant core-genome of the 18 most closely-related genomes including the 2 Beijing *V. cholerae* strains isolated in 2021. (C) Schematic diagram of the CTX prophages identified in *V. cholerae* BJVC202102.

Notes: Branches were colored according to the lineages they belonged to in Figure 1A. The isolation time was labelled and the tips were colored according to the isolation countries in Figure 1B. Schematic diagram of the CTX prophages were identified in *V. cholerae* BJVC202102.

Abbreviations: SNPs=single nucleotide polymorphisms.

Submitted: September 01, 2021; Accepted: December 14, 2021

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Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, November, 2021

Diseases	Cases	Deaths
Plague	0	0
Cholera	0	0
SARS-CoV	0	0
Acquired immune deficiency syndrome*	6,493	2,131
Hepatitis	126,320	35
Hepatitis A	1,026	0
Hepatitis B	102,598	22
Hepatitis C	19,858	10
Hepatitis D	18	0
Hepatitis E	2,055	2
Other hepatitis	765	1
Poliomyelitis	0	0
Human infection with H5N1 virus	0	0
Measles	105	0
Epidemic hemorrhagic fever	2,120	12
Rabies	11	9
Japanese encephalitis	14	1
Dengue	5	0
Anthrax	18	0
Dysentery	2,775	0
Tuberculosis	61,753	128
Typhoid fever and paratyphoid fever	524	0
Meningococcal meningitis	3	0
Pertussis	1,148	0
Diphtheria	0	0
Neonatal tetanus	2	0
Scarlet fever	2,717	0
Brucellosis	3,649	0
Gonorrhea	11,119	0
Syphilis	42,174	4
Leptospirosis	31	0
Schistosomiasis	3	0
Malaria	62	0
Human infection with H7N9 virus	0	0
COVID-19[†]	1,581	0
Influenza	110,691	0
Mumps	11,881	0

Continued

Diseases	Cases	Deaths
Rubella	119	0
Acute hemorrhagic conjunctivitis	2,218	0
Leprosy	28	0
Typhus	155	0
Kala azar	21	0
Echinococcosis	209	0
Filariasis	0	0
Infectious diarrhea[§]	64,241	1
Hand, foot and mouth disease	113,473	0
Total	**565,663**	**2,321**

[*] The number of deaths of Acquired immune deficiency syndrome is the number of all-cause deaths reported in the month by cumulative reported AIDS patients.
[†] The data were from the website of the National Health Commission of the People's Republic of China.
[§] Infectious diarrhea excludes cholera, dysentery, typhoid fever and paratyphoid fever.
The number of cases and cause-specific deaths refer to data recorded in National Notifiable Disease Reporting System in China, which includes both clinically-diagnosed cases and laboratory-confirmed cases. Only reported cases of the 31 provincial-level administrative divisions in the mainland of China are included in the table, whereas data of Hong Kong Special Administrative Region, Macau Special Administrative Region, and Taiwan are not included. Monthly statistics are calculated without annual verification, which were usually conducted in February of the next year for de-duplication and verification of reported cases in annual statistics. Therefore, 12-month cases could not be added together directly to calculate the cumulative cases because the individual information might be verified via National Notifiable Disease Reporting System according to information verification or field investigations by local CDCs.

doi: 10.46234/ccdcw2022.002

Indexed by PubMed Central (PMC), Emerging Sources Citation Index (ESCI), and Chinese Scientific and Technical Papers and Citations

The inauguration of *China CDC Weekly* is in part supported by Project for Enhancing International Impact of China STM Journals Category D (PIIJ2-D-04-(2018)) of China Association for Science and Technology (CAST).

CCDC weekly

Vol. 4 No. 2 Jan. 14, 2022

Responsible Authority
National Health Commission of the People's Republic of China

Sponsor
Chinese Center for Disease Control and Prevention

Editing and Publishing
China CDC Weekly Editorial Office
No.155 Changbai Road, Changping District, Beijing, China
Tel: 86-10-63150501, 63150701
Email: weekly@chinacdc.cn

CSSN
ISSN 2096-7071
CN 10-1629/R1