

REVIEW

SARS-CoV-2 cold-chain transmission: Characteristics, risks, and strategies

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Funding information

National Key Research and Development Program of China, Grant/Award Number: 2021YFC0863000

Abstract

Low temperature and certain humidity are conducive to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) for long-time survival and long-distance spread during logistics and trades. Contaminated cold-chain or frozen products and outer packaging act as the carrier of SARS-CoV-2, that infects the high-risk population who works in the ports, cold storage or seafood market. Since the coronavirus disease 2019 (COVID-19) pandemic worldwide, multiple localized outbreaks caused by SARS-CoV-2 contaminated imported cold-chain products have been reported in China, which brought challenges to COVID-19 prevention and control. Here, we review the evidences of SARS-CoV-2 cold-chain transmission from six confirmed cold-chain related COVID-19 outbreaks in China, especially in terms of SARS-CoV-2 whole-genome sequencing and virus isolation. In addition, we summarize the characteristics and mode of SARS-CoV-2 cold-chain transmission from both six COVID-19 outbreaks in China and the outbreaks suspected cold-chain transmission in other countries. Finally, we analyze the underlying risks of SARS-CoV-2 cold-chain transmission and propose the preventive countermeasures.

KEYWORDS

SARS-CoV-2, cold-chain transmission, COVID-19 outbreak, risk, strategy

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), is mainly transmitted by respiratory droplets and direct contact with

respiratory droplets between humans, and may be transmitted by aerosols when exposed to high concentration of SARS-CoV-2 contaminated aerosols for a long time in a relatively closed environment.^{1,2} Meanwhile, some studies have found that SARS-CoV-2 also transmitted by fomite contact, a more complex

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and hidden transmission route, may introduce SARS-CoV-2 into the low prevalence countries and regions.³⁻⁵

The survival time of SARS-CoV-2 is related to humidity, temperature and other conditions. Low temperature and certain humidity are conducive to the survival and infectivity of the virus,^{3,6} indicating that cold-chain fomites may be more suitable for SARS-CoV-2 long-time survival and long-distance spread during international trades. Since June 2020, SARS-CoV-2 RNA had been detected in imported cold-chain products in most provinces of China.⁷ According to the report of the press conference of Joint Prevention and Control Mechanism of The State Council, a total of 3.23 million samples of cold chain products had been randomly checked by the customs as of October 29, 2021, of which 499 samples were SARS-CoV-2 RNA positive.⁸ In addition, since the localized outbreak of SARS-CoV-2 was brought under control in April 2020, clusters of COVID-19 outbreaks caused by SARS-CoV-2 contaminated imported cold-chain products exposure have occurred in some parts of mainland China, which brought challenges to COVID-19 prevention and control. Therefore, it is necessary to systematically analyze the underlying risks of SARS-CoV-2 transmission by cold-chain products in the ongoing COVID-19 pandemic.

Here, we summarize the characteristics and mode of SARS-CoV-2 cold-chain transmission from six COVID-19 outbreaks caused by SARS-CoV-2 contaminated imported cold-chain products exposure in China and the similar reports of SARS-CoV-2 transmission by cold-chain worldwide. In addition, we analyze the underlying risks of SARS-CoV-2 cold-chain transmission and propose the preventive strategies.

2 | CHARACTERISTICS OF SIX COLD-CHAIN RELATED COVID-19 OUTBREAKS IN CHINA

Up to April 2021, a total of six cold-chain related COVID-19 outbreaks have been confirmed in China. These outbreaks had ruled out the introduction of imported COVID-19 cases according to the field epidemiological investigation, thereby cold-chain products in contact with the initial case were the primary targets for further screening.

2.1 | Evidences overview

According to the investigations of detailed field epidemiology, all six outbreaks were associated with SARS-CoV-2 contaminated imported cold-chain products exposure.⁹⁻¹⁴ The related cold-chain products importers came from the Europe or North America (Table 1). To trace the source of SARS-CoV-2 that caused the outbreak, samples were taken from the surface of the initial or index cases associated cold-chain products each outbreak, and were subjected to SARS-CoV-2 specific RT-PCR testing. Although the positive rates of SARS-CoV-2 RNA varied greatly (0.8%–40.7%), SARS-CoV-2 RNA was detected

from the surface samples of cold-chain products in all six outbreaks. SARS-CoV-2 RNA was found on the surface of frozen food in two outbreaks and that in the outer packaging of frozen products in other four outbreaks.

The key evidences of six COVID-19 outbreaks were marked in Figure 1, including the presence of complete field epidemiological evidence, the relevance of SARS-CoV-2 full-length genomes of cold-chain products to the initial cases, as well as the successful isolation of alive SARS-CoV-2 from initial case associated cold-chain products. Among that, the relevance of SARS-CoV-2 full-length genomes divided into three degrees: (I) Complete identical virus means that the genome of SARS-CoV-2 from the cold-chain products same as the one from initial case; (II) Former generation virus means that the genome of SARS-CoV-2 from the cold-chain products lacked one nucleotide mutation than the one from the initial case; (III) Highly homologous virus means that the genome of SARS-CoV-2 from the cold-chain products shared key nucleotide mutations with the one from the initial case.

All six outbreaks had the evidences of epidemiological investigation. Five outbreaks had the different degree evidences of SARS-CoV-2 genomes relevance of cold-chain products to the initial cases. Live SARS-CoV-2 was successful isolated in the first Qingdao COVID-19 outbreak. Nonetheless, the full-genome sequence of SARS-CoV-2 was not obtained in Tianjin COVID-19 outbreak due to low viral load of the samples from cold-chain products.

2.2 | Evidence based on SARS-CoV-2 whole-genome sequencing

Considering the difficulty for isolation of live virus, genome sequencing played the key role in virus source tracing. According to the databases of Chinese Center for Disease Control and Prevention and Global Initiative on Sharing All Influenza Data (GISAID, <https://www.gisaid.org/>), viral genome sequence alignment analyses showed that the SARS-CoV-2 strains that caused six outbreaks had no relationship to the previous Chinese local COVID-19 outbreaks, whereas some of these strains were highly homologous with the circulating strains abroad, including the Europe or North America (Figure 2), which were consistent with the original countries of cold-chain products imported from the outbreak area.

In Beijing Xinfadi market COVID-19 outbreak, only highly homologous virus genome sequence was obtained as the insufficient sequencing coverage of cold-chain products. The SARS-CoV-2 genome sequence in the surfaces of frozen salmon in Beijing Xinfadi market had at least three mutation sites consistent with the SARS-CoV-2 infected by the initial case (Table 1). Limited viral genomic evidence suggests an association with SARS-CoV-2 contaminated cold-chain products. While in the first outbreaks of Dalian and Qingdao, the genomes of SARS-CoV-2 with one less mutation were identified in the surface of frozen products than in the initial case, indicating a direct correlation that the parental virus in cold-chain products infected

TABLE 1 Characteristics of cold-chain-related COVID-19 outbreaks in China

Outbreak city	Positive ratio (%)	Source of the virus	Original continent of cold-chain	Pango lineage	Mutated nucleotide no.			Occupation of index case	Exposed place	Duration (days)	Infected cases no.	Affected provinces
					Initial/ index case	Cold-chain environment	Shared					
Beijing	0.8 (2/242)	Surface of frozen salmon	-	B.1.1	8	≥3	3	Seafood market staff	Seafood market	37	436	Hebei, Liaoning, Sichuan, Tianjin, Zhejiang, Henan
Dalian-1st	40.7 (134/329)	Outer packing of frozen cod	Europe	B.1.1.1317	14	13	13	Seafood processing staff	Seafood processing workshop	15	152	Jilin, Beijing, Heilongjiang, Fujian
Qingdao-1st	12.1 (51/420)	Outer packing of frozen cod	Europe	B.1.1	13/14	12/13	12	Dockworker	Dock	36	15	-
Tianjin	6.6 (8/121)	Surface of frozen pig head	North America	B.1.413	9	-	-	Stevedore	Cold storage	1	2	-
Qingdao-2nd	3.1 (12/388)	Outer packing of frozen cod	Europe	B.1.1	15	15	15	Dockworker	Seafood processing workshop	2	2	-
Dalian-2nd	1.1 (13/1170)	Outer packing of frozen fish	Europe	B.1.1	12	12	12	Dockworker	Dock	25	83	-

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aPositive rate of SARS-CoV-2 RNA in the samples of related cold-chain products.

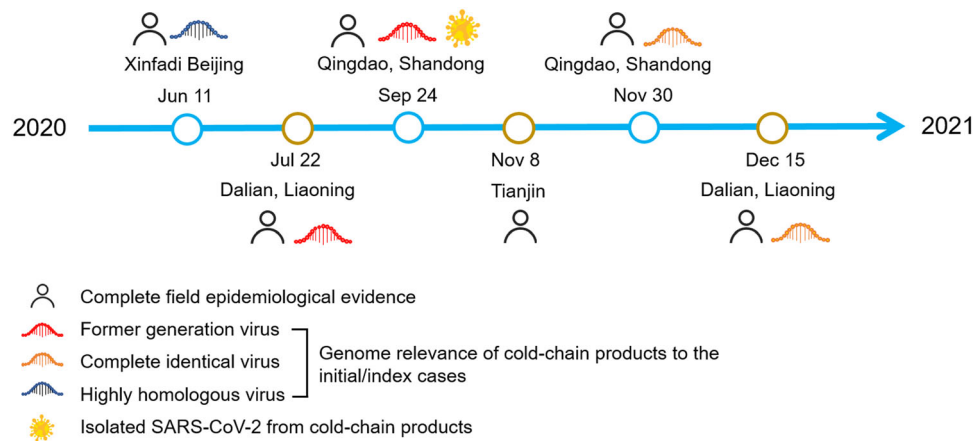


FIGURE 1 Timeline of cold-chain related COVID-19 outbreaks. The key evidences of cold-chain related COVID-19 outbreaks were indicated by corresponding symbols above/below the corresponding outbreak. COVID-19, coronavirus disease 2019

the initial cases. Furthermore, live parental SARS-CoV-2 was successfully isolated in the first cold-chain related COVID-19 outbreak of Qingdao and the genome sequencing showed 100% nucleic acid identity with the initial case (Figure 2), confirming that the SARS-CoV-2 in cold-chain environments were alive and had ability to infect human who exposed to.

In the second cold-chain related COVID-19 outbreaks of Dalian and Qingdao, the genomes of SARS-CoV-2 on the surface of frozen products had the 100% genomic identity as the viruses infected by the initial or index cases. For these two outbreaks, it is hard to determine the source of the virus depending on the viral genomic evidence because of the possibility that detected virus/viral nucleic acids on the surfaces of goods/packages were contaminated by the patients in the working environment. Indeed, China has carried out routine SARS-CoV-2 RNA screening and implemented strict closed-loop management for cold-chain porters, thus the probability of infected person exist before contact the cold chain products is low. Therefore, combined with the viral genomic evidence, it was confirmed that the SARS-CoV-2 viruses caused the two outbreaks originated from the imported cold chain products.

It's worth noting that ruling out the possibility of SARS-CoV-2 shedding from patients or asymptomatic SARS-CoV-2 carriers in the outbreaks to contaminate associated cold-chain environments was the first prerequisite for confirming the transmission of SARS-CoV-2 from cold-chain products to humans. In addition to rigorous SARS-CoV-2 RNA screening and strict closed-loop management, the alignment and analysis of viral genome acquired from the surface of cold-chain products and first-episode patients plays an important role in outbreak tracing. After human infected with SARS-CoV-2, the viruses may have evolved during the replication process to adapt to the body immune pressure.¹⁵ Consistent with the pattern, the viral genome data from the above COVID-19 outbreaks showed that the number of nucleotide mutations of SARS-CoV-2 sequences in the initial/index cases were more than or equal to those in the cold-chain products.

Therefore, the evidences from whole-genome sequencing plus the field epidemiological investigation amply clarified the correlation

between the SARS-CoV-2 of the initial case and the cold-chain products, indicating that the cold-chain products can act as carriers for SARS-CoV-2, introducing the virus into new areas and causing subsequent transmission.

2.3 | Epidemiological features

All six COVID-19 outbreaks have obviously epidemiological features. The outbreak in Beijing Xinfadi Market in June 2020 was the longest duration, largest cases and widest scale of the six outbreaks because of the magnification by the working environment of the cold chain/seafood stall and the floating population in the market (Table 1). And it is the only outbreak that the site of the initial case exposure was in seafood wholesale market and the initial/index cases were consumers. Except for Beijing, other cities were all located in the coastal area of Bohai Bay in China (Figure 3), meanwhile the COVID-19 outbreaks in two cities (Dalian and Qingdao) had occurred two times. The initial/index cases were dockworkers or seafood processing staff who had direct contact with SARS-CoV-2 contaminated cold-chain products, showing obvious occupations clustered and the sites that the initial cases exposure were concentrated in frozen aquatic products processing workshops or docks.

3 | OUTBREAKS LINKED TO COLD-CHAIN OR FROZEN FOODS IN OTHER COUNTRIES

Except for China, 4 countries had reported the cold-chain or frozen foods related outbreaks. From April to May 2020, there had been 16 233 cases among workers from 239 meat and poultry processing industries in 23 states of the United States infected and 86 of whom died.¹⁶ From March to July 2020, Ireland reported 23 outbreaks of COVID-19 in meat processing plants, with 1047 cases among workers.¹⁷ On 19 July 2020, the United Kingdom reported an



FIGURE 2 (See caption on next page)

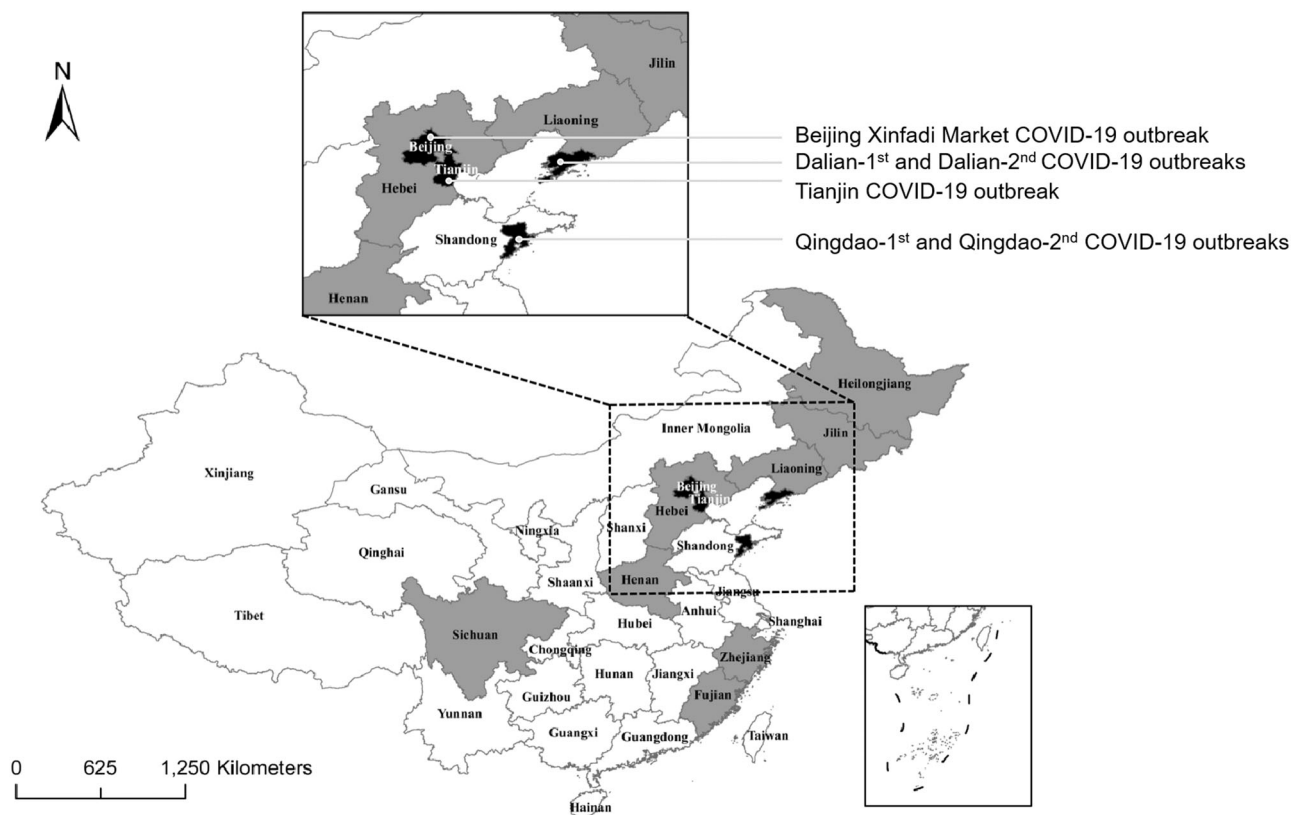


FIGURE 3 Geographical distribution of cold-chain-related COVID-19 outbreaks in China. Dark gray: provinces associated with cold-chain related COVID-19 outbreaks, light gray: provinces affected by the cold-chain related COVID-19 outbreaks. COVID-19, coronavirus disease 2019

outbreak of COVID-19, and the cases involving more than 100 workers at several meat processing plants in the United Kingdom.¹⁸ On July 20, 2021, a Vietnamese journalist published a news on Seafood Source about an outbreak of COVID-19 in Singapore with 700 cases involved and finally forced the closure of The Jurong Fish Port, which is the largest wholesale seafood market in Singapore.¹⁹

The main reasons for the outbreaks of meat processing plants were the particularity of the workplace, the inability of maintaining social distance, the failure of strictly observe hand hygiene and poor ventilation. Despite the frequent occurrence of COVID-19 outbreaks in meat and poultry cold-chain processing plants, the large-scale epidemic of COVID-19 in local communities make it hard to perform precise virus source tracing. However, these reports confirmed that the viral shedding from the infected workers can make the food or the packaging contamination in the meat processing industry.

Although the SARS-CoV-2 is unlikely to cause an epidemic through foodborne transmission, the cold-chain especially the frozen foods may present a systematic risk in the ongoing COVID-19 pandemic because of the SARS-CoV-2 remains highly stable under refrigerated at 4°C and freezing conditions.²⁰

4 | TRANSMISSION MODE OF SARS-COV-2 IN COLD-CHAIN RELATED COVID-19 OUTBREAKS

According to the analyses of the characteristics of six cold-chain related outbreaks and the cold-chain transmission data worldwide, the transmission mode via the cold-chain fomite has been preliminary summarized. Cold-chain products and outer packaging can be contaminated with SARS-CoV-2 during processing, packaging and

FIGURE 2 Phylogenetic analysis of SARS-CoV-2 based on the representative full-length genome sequences of the cold-chain related COVID-19 outbreaks in China. Strains associated with the six outbreaks in China were indicated by red on the corresponding right. The sequences of index case, initial case and corresponding environment from each cold-chain related COVID-19 outbreaks were highlighted in various colors. The strains from the Europe and North America showed highly homologous with the local outbreak strains were indicated by black dots. The genome of the reference COVID-19 from Wuhan in December 2019 was shaded in grey. The PANGO lineages were marked and colored on the right. The tree was rooted using strain WH04 (EPI_ISL_406801) in accord with the root of PANGO tree. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

transportation. Once the cold-chain products arrived the destination, SARS-CoV-2 may infect high-risk population through direct contact with the products. Subsequently, the infected high-risk people further transmit the SARS-CoV-2 in communities through human-to-human transmission, leading to COVID-19 outbreaks. The high-risk population of SARS-CoV-2 cold-chain transmission is those who are working in the ports, cold storage and seafood markets, including dockers and porters, cold-chain processing workers and seafood market salesmen. The hands, faces, clothing of high-risk people without effective protection direct touch SARS-CoV-2 contaminated surface of the cold-chain products during the processes of handling, loading and unloading, and then further infected through irregular behavior such as touching the mucous membrane of mouth, nose and eyes. In addition, specific locations, such as seafood market stalls and sealed cold-chain repackaging plants, also played a role in amplifying the epidemic.

The World Health Organization-China joint research group reported that the proportion of cases related to stalls with cold-chain products in Huanan Seafood Market was significantly higher than that of stalls without cold-chain products with a relative infection risk by 3.3 times as high. Furthermore, of the 21 stalls that tested positive for SARS-CoV-2 RNA in environment samples, 16 stalls sold cold-chain products.²¹ These results highly indicate that there is a possibility of SARS-CoV-2 entering Wuhan Huanan Seafood Market through frozen food at the end of 2019 and causing the epidemic.

5 | UNDERLYING RISKS AND PREVENTIVE STRATEGIES OF SARS-COV-2 TRANSMISSION BY COLD-CHAIN PRODUCTS

According to the analysis of the six cold-chain related COVID-19 outbreaks in China, the imported cold chain products is one of the important causes for the recurrence of COVID-19. There are two underlying risks to the cold-chain fomite transmission for prevention and control of the COVID-19 epidemic. One is cold-chain fomite including the rapid international logistics under low temperature will greatly accelerate the global spread of SARS-CoV-2, especially in areas where the COVID-19 epidemic is well controlled.^{22,23} The other is "old strains" circulating in the past that stored in cold storage could be reactivated, causing "silent transmission" and triggering new COVID-19 outbreaks, and the latter may further affect the evolutionary process of SARS-CoV-2 and pose a threat to future prevention and control as well as immunization strategies. Therefore, formulating a comprehensive prevention strategy to deal with the localized outbreak epidemic at any time should be considered as a priority for public authorities worldwide.

China has gradually improved the prevention and control strategies^{24,25}: (I) Strengthening the sampling inspection of imported cold-chain products to find the source of infection in a timely manner; (II) Developing disinfectant and disinfection

equipment in low-temperature to comprehensively disinfect imported cold-chain products and cut off transmission routes; (III) Enhancing vaccination, personal protection training and health monitoring for the protection of high-risk population; (IV) Developing the technical guidelines for ranking and classifying SARS-CoV-2 contaminated cold-chain foods; (V) Improving the sampling strategies for tracing the cold-chain source. Indeed, following the first Dalian COVID-19 outbreak in July 2020, the cold-chain related outbreaks in China were limited to the cities importing cold chain products and did not spread to other provinces. However, with tens of millions of imported cold-chain products in China, it is not realistic to completely block the introduction of SARS-CoV-2, but the implementation of these strategies will minimize the introduction and prevalence of the virus.

Effectively capturing the cold-chain introduced SARS-CoV-2 still requires two necessary conditions: First, it is realistic to trace the source of every localized COVID-19 outbreak in countries and regions where cases have been eliminated or the epidemic has been interrupted; The other is to have a professional tracing investigation team, with epidemiological investigation, nucleic acid testing, serum antibody detection and virus genome sequencing and other technical capabilities. At present, SARS-CoV-2 is still pandemic worldwide, in which human-to-human transmission remains the dominant mode of SARS-CoV-2 transmission.²⁶ Therefore, not all countries have the capability or necessary to capture SARS-CoV-2 introduced by the cold chain. Nevertheless, it doesn't mean that the transmission of cold-chain fomites should be overlooked and the cold-chain products is one of the most important ways for the outbreak and spread of COVID-19. Because the imbalance of global epidemic control and vaccine levels, blocking the SARS-CoV-2 cold-chain introduction and transmission may be what most countries of the world must go through before ending the COVID-19 pandemic.

6 | CONCLUSION

The cold-chain related outbreaks of COVID-19 in China provides persuasive evidence that cold-chain products and packaging act as the carrier to introduce SARS-CoV-2 that cause local transmission in the ports, cold storage or seafood markets, and even leading to community outbreak if not well-controlled. The international community should pay close attention to the SARS-CoV-2 cold-chain transmission, strengthen the disinfection of cold-chain products and the personnel protective measures in aspect of the production, processing, transportation and sale of cold-chain products, preventing SARS-CoV-2 reintroduced or silent transmission by cold-chain.

AUTHOR CONTRIBUTIONS

Jun Han and Wenbo Xu: conceived and supervised the study. **Ying Xia and Zhixiao Chen, Xiang Zhao, Ji Wang, Kai Nie and Peihua Niu:** collected the data and performed analysis. **Cao Chen and Yanan Feng:** drafted and revised the manuscript. All authors consented for the final draft.

ACKNOWLEDGMENTS

We would like to express sincere thanks to the staffs of Beijing, Dalian, Tianjin and Qingdao for their contribution to epidemic prevention and control. This study was supported by the National Key R&D Program of China (2021YFC0863000).

CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study were kept by National Institute for Viral Diseases Control and Prevention, China CDC. Please contact author for data requests.

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How to cite this article: Chen C, Feng Y, Chen Z, et al. SARS-CoV-2 cold-chain transmission: Characteristics, risks, and strategies. *J Med Virol*. 2022;94:3540-3547. doi:10.1002/jmv.27750