Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Dynamic changes of serum SARS-CoV-2 antibody levels in COVID-19 patients

Dear Editor,

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in December 2019 and spread rapidly and globally, leading to a worldwide pandemic currently. As of April 4, 2022, more than 489 million confirmed COVID-19 cases and 6 million deaths were reported to WHO around the world.[1] Evaluating the durability of the humoral immune response to SARS-CoV-2 is essential to understand the susceptibility of COVID-19 patients to the same virus after recovered from the infection. Recently, it has been reported in neutralization assays using SARS-CoV-2 virus that neutralization antibodies titer is correlated with protection against re-infection.[2] Serum immunoglobulin M (IgM) and immunoglobulin G (IgG) against SARS-CoV-2 were detectable within the first several weeks after symptom onset.[3] To date, there are few data on long-term of antibodies response to SARS-CoV-2 after initial infection. Here, the aim of this study is to investigate the duration or stability of viral-specific humoral responses in COVID-19 recovered patients.

This was a retrospective study involving 61 hospitalized patients with laboratory-confirmed COVID-19 in the Renmin Hospital of Wuhan University, Wuhan, China from January 17, 2020 to March 7, 2020. Patients received follow-up visits form February 25, 2020 to January 29, 2021. Each patient had at least two antibody tests, with a total of 173 serum samples collected. The time points were defined as the days after symptom onset in which samples were collected. From the first to fourth time point, it was days 1–30, 31–60, 61–120, and 296–368, respectively. The serum antibodies against SARS-CoV-2 (IgM, IgG and neutralization antibodies) were detected by a chemiluminescent immunoassay (YHLO Biotech, Shenzhen, China). The samples were processed and analyzed according to the manufacturer's instructions. Values $\geq$ 10 AU/mL are considered positive. This study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (Approve No. WDRY2020-K073). Continuous variables were present as median and interquartile range (IQR) and the Kruskall-Wallis test was applied. A p-value less than 0.05 was statistically significant. All statistical analyses and scientific graphics were made by using SPSS 20.0.

From 17 January 2020 to 7 March 2020, a total of 61 COVID-19 patients were recruited in our study. The median age was 34 years (IQR, 30–47). 72.13% (44/61) were women and 27.87% (17/61) men. 29, 53, 30 and 61 specimens were collected 1–30 days, 31–60 days, 61–120 days, and 296–368 days after the onset of symptoms, respectively. We illustrate the overall profile of serum IgM, IgG, and neutralization antibodies against SARS-CoV-2 from day 1 to 368 after illness onset in Table 1 and Fig 1.

The median serum IgM level was 22.79 (IQR, 6.45, 150.97) AU/mL in 1–31 days, reduced to 17.61 (IQR, 3.65, 57.05) AU/mL in 31–60 days, increased to 31.58 (IQR, 11.38,125.22) AU/mL in 61–120 days, but the difference was not statistically significant. The IgM level in days 296 to 368 reduced to 2.66 (IQR, 1.16, 8.22) AU/mL, which decreased significantly compared with other time periods (Fig. 1). The positive rates of IgM were 65.52%, 58.49%, 80.00% and 22.95%, respectively (Fig. 2).

The median serum IgG level was 86.67 (IQR, 46.88, 208.04) AU/mL in 1–31 days, increased to 123.67 (IQR, 56.22, 191.01) AU/mL in 31–60 days and 158.94 (IQR, 115.69, 232.16) AU/mL in 61–120 days, but the difference was not statistically significant. The IgG level in days 296 to 368 reduced to 36.32 (IQR, 14.68,78.68) AU/mL, which decreased significantly compared with other time periods (Fig. 1). The positive rates of IgG were 96.55%, 100.00%, 100.00% and 81.97%, respectively (Fig 2).

The median serum neutralization antibodies level was 35.32 (IQR, 12.16, 160.60) AU/mL in 1–31 days, increased to 54.43 (IQR, 23.98, 145.74) AU/mL in 31–60 days and 80.13 (IQR, 56.98, 202.36) AU/mL in 61–120 days, but the difference was not statistically significant. The neutralization antibodies level in days 296 to 368 markedly reduced to 14.30 (IQR, 7.77, 27.9) AU/mL, which decreased significantly compared with other time periods (Fig. 1). The positive rates of neutralization antibodies were 79.31%, 92.45%, 100.00% and 67.21%, respectively (Fig. 2).

Some studies performed at the beginning of the COVID-19 pandemic showed that the amount of anti-SARS-COV-2 IgM/IgG antibodies decreased in several months post onset of symptoms.[4,5] However, Gong X et al. found that anti-SARS-CoV-2 IgG can last for at least 9 months in patients with a history of natural infection.[6] Kaygusuz S et al. also showed that both IgG and neutralization antibodies levels continued unabated after 9 months of follow-up.[7] Yousefi Z et al. found that the level of anti-SARS-CoV-2 IgG antibody was detectable at their highest level for 3 months, and a certain amount of anti-SARS-COV-2 IgG antibody could be detected in the serum of recovered patients up to 15 months.[8] The humoral immunity persisted for up to 18 months in patients with mild COVID-19.[9] The maximum duration of neutralizing antibodies and IgG antibodies could be long-lasting based on Linear Mixed Models, especially IgG.[10]

Our study has several limitations such as small sample size, retrospective study, and different sample collection time. The relationship between clinical features and antibody dynamics, such as disease severity, was not discussed.

According to the present study's results, despite the decrease in the amount of IgG and neutralization antibodies in 297–368 days,

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Table 1
Levels of serum antibodies at different times.

	1–30 (d)	31–60 (d)	61–120 (d)	296–368 (d)
IgM antibodies	22.79 (6.45, 150.97)	17.61 (3.65, 57.05)	31.58 (11.38, 125.22)	2.66 (1.16, 8.22)
IgG antibodies	86.67 (46.88, 208.04)	123.67 (56.22, 191.01)	158.94 (115.69, 232.16)	36.32 (14.68, 78.68)
Neutralization antibodies	35.32 (12.16, 160.60)	54.43 (23.98, 145.74)	80.13 (56.98, 202.36)	14.30 (7.77, 27.9)

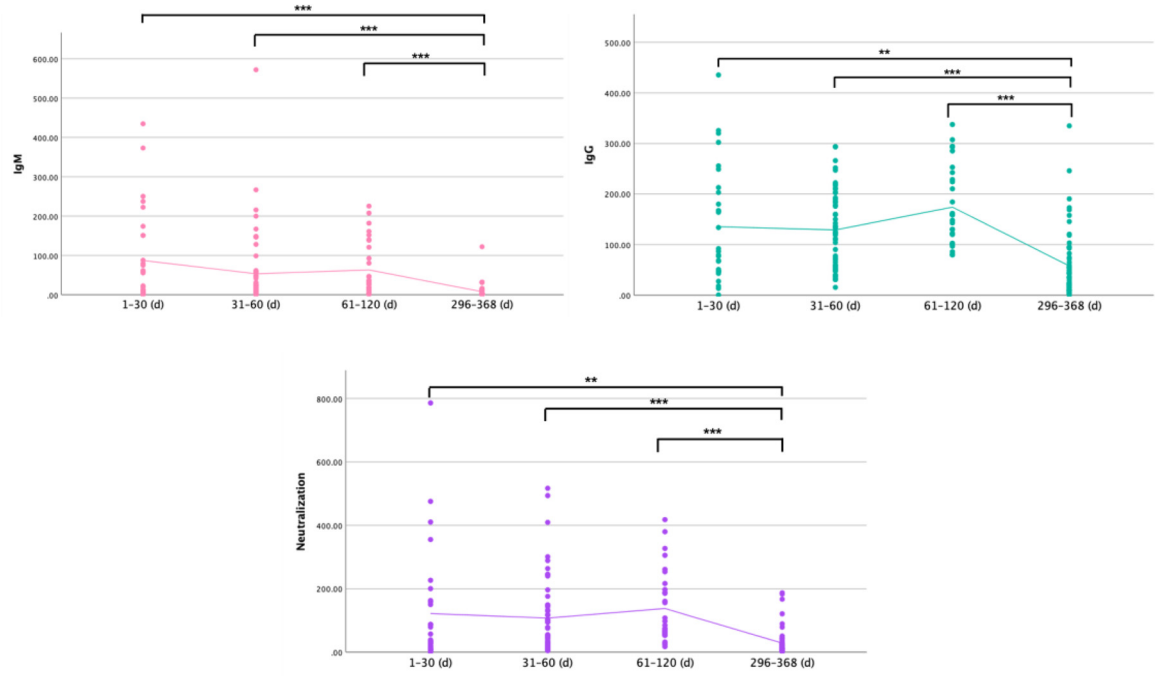

Fig. 1. Dynamic changes in antibodies against SARS-CoV-2. (A–C) Dynamic changes in IgM antibody (A), IgG antibody (B) and neutralization antibodies (C) in representative patients over the monitoring period. P-values were calculated by Kruskal Wallis test. P-values < 0.05 were considered significant with *, **, and *** indicate $p < 0.05$, <0.01, and <0.001, respectively.

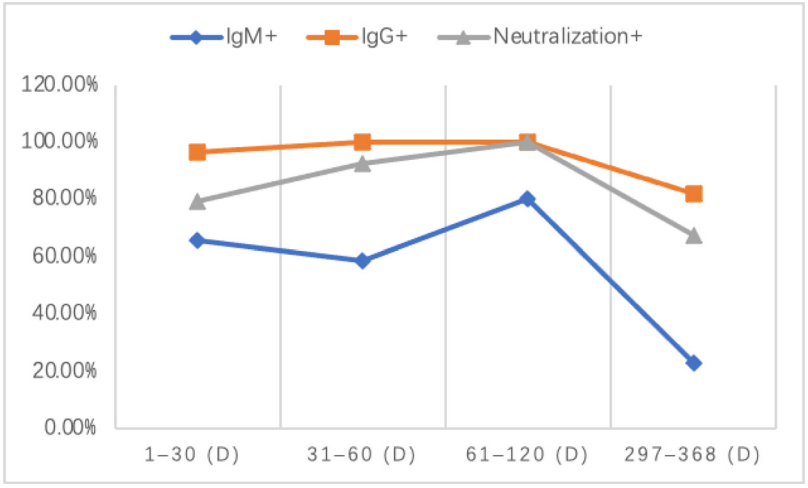

Fig. 2. Serum antibody positive rates at different times. The positive rates of IgM, IgG and neutralization antibodies reached the peak in 61–120 days and decreased significantly in 297–368 days.

a certain amount of antibody could still be detected in most recovered COVID-19 patients.

References

1. WHO Coronavirus (COVID-19) Dashboard[EB/OL]. 2022. https://covid19.who.int/.
2. Khoury D.S., Cromer D., Reynaldi A., et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**(7):1205–11.
3. Zhang W., Du R.H., Li B., et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 2020;**9**(1):386–9.
4. Figueiredo-Campos P., Blankenhaus B., Mota C., et al. Seroprevalence of an-

ti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. *Eur J Immunol* 2020;**50**(12):2025–40.
5. Ma H., Zhao D., Zeng W., et al. Decline of SARS-CoV-2-specific IgG, IgM and IgA in convalescent COVID-19 patients within 100 days after hospital discharge. *Sci China Life Sci* 2021;**64**(3):482–5.
6. Gong X., Cui P., Wu H., et al. Long-term immune responses in patients with confirmed novel coronavirus disease-2019: a 9-month prospective cohort study in Shanghai, China. *BMC Infect Dis* 2022;**22**(1):240.
7. Kaygusuz S., Korukluoğlu G., Coşgun Y., et al. Investigation and long-term monitoring of the presence of neutralizing antibody in patients with covid-19 disease of different clinical severity. *J Med Virol* 2022.
8. Yousefi Z., Taheri N., Dargahi M., et al. Long-term persistence of anti-SARS–COV-2 IgG antibodies. *Curr Microbiol* 2022;**79**(4):96.
9. Choe P.G., Hong J., Park J., et al. Persistent antibody responses up to 18 months after mild SARS-CoV-2 infection. *J Infect Dis* 2022.
10. Guzmán-Martínez O., Guardado K., Varela-Cardoso M., et al. Generation and persistence of S1 IgG and neutralizing antibodies in post-COVID-19 patients. *Infection* 2021:1–10.

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Reduced neutralizing antibody response in naïve Covishield vaccinees against Omicron emphasizes booster vaccination

Dear Editor,

In this journal, Yang et al., demonstrated significant increase in neutralizing antibody response against Alpha, Beta, Gamma, Delta and Omicron variant post homologous booster vaccination of BBIBP-CorV.[1] Many studies have proven the importance of regular and booster vaccination in protecting the human population from serious disease and mortality from SARS-CoV-2. India has severely affected with emerging SARS-CoV-2 Variants of Concern (VOCs) during several waves of the COVID-19 pandemic. In fight against SARS-CoV-2, India has initiated national COVID-19 vaccination program on 16 January 2021. A major part of the population in India have been administered with the first/second dose of Covishield vaccine (1,51,12,99,993). However, small population has received the first/second dose of Covaxin, an indigenously developed inactivated vaccine (30,69,67,102). Even with the high vaccination coverage in the country, large number of the breakthrough cases were reported and created hesitancy for vaccination. The waning immune response post vaccination is the probable cause and important factor associated with these breakthrough infections.

The recently emerged highly transmissible SARS-CoV-2 Omicron variant has also caused exponential rise in Covid-19 cases with breakthrough infection and re-infection worldwide including India.[2-5] The Omicron variant have been evolved into 75 sub lineages. These sub lineages have been detected from 175 countries and BA.1.1, BA.2, BA.1 found to be dominating the other sub lineages of Omicron.[6] Recently, the World health organization has also warned about XE variant which is mutant hybrid of BA.1 and BA.2. The Omicron has also altered pathogenesis due to change in cellular tropism which could be the reason for the milder disease.[7] It has been reported that the Omicron variant significantly evades immune response generated with therapeutic monoclonal antibodies and the vaccine-elicited neutralizing antibodies after two doses of COVID-19 vaccine.[8] Andrews and Rossler et al., demonstrated limited protection against severe disease caused by the Omicron variant in individuals vaccinated with two doses of ChAdOx1 nCoV-19.[9-10] Besides this, few studies have also reported reduced neutralization of Omicron variant with the sera of ChAdOx1 nCoV-19 vaccinees.[11-12] Apparently, it has been found that the booster dose of mRNA vaccine boosts the immune response which protects against Omicron but immunity wanes over time.[13]

Globally, various research groups are studying the vaccine effectiveness against the VOCs and the rate of breakthrough post vaccination. Here, we report the IgG and neutralizing antibody response in individuals vaccinated with two doses of Covishield vaccine against B.1, Delta, Beta and Omicron variant.

Briefly, the sera were collected from COVID-19 naïve individuals vaccinated with two doses of Covishield (n = 24; 180 days post second vaccination), COVID-19 recovered cases vaccinated with two doses of Covishield (n = 17; 180 days post second vaccination) and individuals with SARS-CoV-2 breakthrough infection post vaccination with two doses of Covishield (n = 46; 14–30 days post infection). All the recovered cases (n = 17) were infected with prototype B.1 variant. Of the 46 breakthrough cases, complete genome could be retrieved only from 21 cases. Seventeen cases were found to be affected with Delta variant, while four with Kappa variant. All the serum samples were screened for IgG antibodies using S1-RBD and N-protein ELISA. Besides this, the neutralization potential of these sera was assessed against B.1, Delta, Beta and Omicron variant with plaque reduction neutralization test (PRNT).

The geometrical mean titre (GMT) of IgG antibodies with S1-RBD ELISA was lesser in naïve vaccinees (147.5; 95% CI: 92.4–235.4) compared to the recovered cases (634.2; 95% CI: 406.1–990.6) and breakthrough cases (1683; 95% CI: 1119–2553). Similar decreasing trend in GMT titres was observed with N protein ELISA for naïve vaccinees (77.2; 95% CI: 55.1–109.6) with respect to recovered cases (112.1; 95% CI: 69.9–179.9) and breakthrough infection (594.1; 95% CI: 349.9–1009) (Fig. 1A-B).

Neutralization studies demonstrated reduction in the GMT titre of neutralizing antibodies (NAb) against Omicron with sera of naïve vaccinees (32.81-fold; 95% CI: 14.7–73.2), recovered cases (28.13-fold; 95% CI: 75.9–10.4) and breakthrough cases (46.86-fold; 95% CI: 74.1–29.6) compared to prototype B.1 variant. All the three groups effectively neutralized the B.1, Beta and Delta variants than

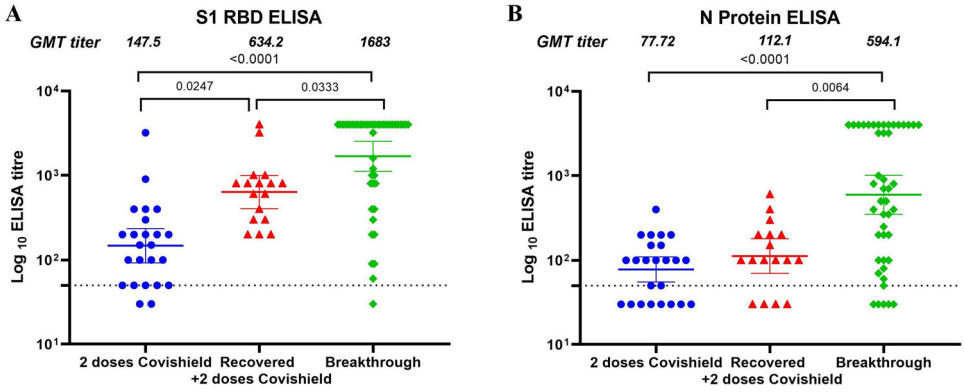

Fig. 1. Anti SARS-CoV-2 IgG antibody response in sera of individuals vaccinated with two doses of Covishield (blue), recovered cases with Covishield vaccination (red) and breakthrough cases with Covishield vaccination (green) (A) IgG antibody response with S1-RBD ELISA (B) IgG antibody response with N protein ELISA. A two-tailed pair-wise comparison was performed using the Kruskal Wallis test with a p-value of 0.05. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as geometric mean titer values with 95% confidence interval.

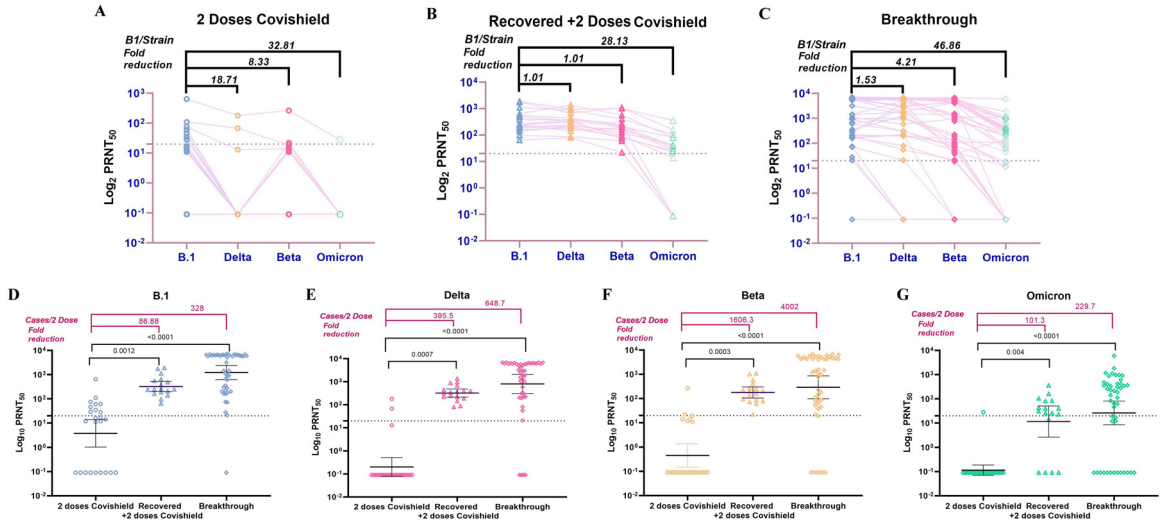

Fig. 2. Neutralizing antibody (NAb) response in sera of individuals vaccinated with (A) two doses of Covishield; (B) recovered cases with Covishield vaccination and (C) breakthrough cases with Covishield vaccination against Delta, Beta and Omicron variants compared to B.1. NAb tires in three groups against (D) B.1, (E) Delta, (F) Beta, (G) Omicron variants with respect to vaccinated individuals with two doses. A two-tailed pair-wise comparison was performed using the Kruskal Wallis test with a p-value of 0.05. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as geometric mean titer values with 95% confidence interval.

Omicron (Fig. 2A-G). The GMT titre of NAb was lowest for Omicron with the sera of naïve vaccinees (0.11; 95% CI: 0.07–0.19) than the recovered cases (11.28; 95% CI: 2.63–51.1) and breakthrough cases (26.25; 95% CI: 8.5–81.4) (Fig. 2G). With highest mutations in spike region, omicron variant clearly showed immune escape against respective neutralizing antibodies induced with Delta variant infection in breakthrough cases than B.1, Delta and Beta variant. Even though highest fold-reduction amongst breakthrough cases was observed with Omicron variant, it also had highest NAb titre than the recovered cases and naïve vaccinees. Breakthrough cases also represented maximum NAb response against all the variants than naïve vaccines and recovered cases.

Despite the fact that vaccines are effective against severe SARS-CoV-2 infection, many breakthrough and re-infection cases were observed during the pandemic. The shifting paradigm would be mainly due to the reducing/waning immune responses post either natural infection or vaccinations, emergence of the new SARS-CoV-2 variant and its immune escape potential. In summary, our study demonstrated lowest IgG and NAb response in naïve vaccinees than other groups. This emphasizes the waning immune response in naïve vaccinees post second dose and warrants the administration of precautionary dose to boost the immunity.

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Conflicts of Interest

Authors do not have a conflict of interest among themselves.

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References

1. Yang Y., Gong X., Yang L., Li J., Zhang J., Wei L., Ye G., Tang Y., Jiang Y., Li J., Lin Y. Regular and booster vaccination with inactivated vaccines enhance the neutralizing activity against Omicron variant both in the breakthrough infections or vaccinees. *J Infect* 2022 Jan 10.
2. Kuhlmann C., Mayer C.K., Claassen M., Maponga T.G., Sutherland A.D., Suliman T., Shaw M., Preiser W. Breakthrough infections with SARS-CoV-2 Omicron variant despite booster dose of mRNA vaccine. Available at SSRN 3981711. 2021 Dec 9.

3. Maruki T., Iwamoto N., Kanda K., Okumura N., Yamada G., Ishikane M., Ujiie M., Saito M., Fujimoto T., Kageyama T., Saito T.. Two cases of breakthrough SARS–CoV-2 infections caused by the Omicron variant (B. 1.1. 529 lineage) in international travelers to Japan. *Clin Infect Dis* 2022 Jan 3.
4. Mohapatra R.K., Tiwari R., Sarangi A.K., Sharma S.K., Khandia R., Saikumar G., Dhama K.. Twin combination of Omicron and Delta variant triggering a Tsunami wave of ever high surges in COVID-19 cases: a challenging global threat with a special focus on Indian sub-continent. *J Med Virol* 2022 Jan 10.
5. Chen J., Wang R., Gilby N.B., Wei G.W.. Omicron variant (B. 1.1. 529): Infectivity, vaccine breakthrough, and antibody resistance. *J Chem Inf Model* 2022 Jan 6.
6. Latif Alaa Abdel, Mullen Julia L., Alkuzweny Manar, Tsueng Ginger, Cano Marco, Haag Emily, Zhou Jerry, Zeller Mark, Hufbauer Emory, Matteson Nate, Wu Chunlei, Andersen Kristian G., Su Andrew I.Omicron Variant Report Karthik Gangavarapu, Laura D. Hughes, and the Center for Viral Systems Biology. *outbreak.info* 2 May 2022. available at https://outbreak.info/situation-reports/omicron Accessed.
7. Bo Meng, Ferreira I., Abdullahi A., Goonawardane N., Saito A., Kimura I., et al. SARS-CoV-2 Omicron spike mediated immune escape and tropism shift. *bioRxiv* 2021;**doi** 12.17.473248. doi:10.1101/2021.12.17.473248.
8. Cao Y., Wang J., Jian F., et al. Omicron escapes the majority of existing SARS–CoV-2 neutralizing antibodies. *Nature* 2022;**602**:657–63.
9. Andrews N., Stowe J., Kirsebom F., Toffa S., Rickeard T., Gallagher E., Gower C., Kall M., Groves N., O'Connell A.M., Simons D. Covid-19 vaccine effectiveness against the omicron (B. 1.1. 529) variant. *N Engl J Med* 2022 Mar 2.
10. Rössler A., Riepler L., Bante D., von Laer D., Kimpel J.. SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons. *N Engl J Med* 2022 Jan 12.
11. Medigeshi G.R., Batra G., Murugesan D.R., Thiruvengadam R., Chattopadhyay S., Das B., Gosain M., Singh J., Anbalagan A., Shaman H., Pargai K.. Sub-optimal Neutralisation of Omicron (B. 1.1. 529) Variant by Antibodies induced by Vaccine alone or SARS-CoV-2 Infection plus Vaccine (Hybrid Immunity) post 6-months. *medRxiv* 2022 Jan 1.
12. Yadav P.D., Sapkal G.N., Sahay R.R., Potdar V.A., Deshpande G.R., Patil D.Y., Nyayanit D.A., Shete A.M., Shastri J., Awate P., Malhotra B.. Substantial immune response in Omicron infected breakthrough and unvaccinated individuals against SARS-CoV-2 variants of concern. *J Infect* 2022 Jan 1.
13. Shen X.. Boosting immunity to Omicron. *Nat Med* 2022;**24**:1–2.

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Association of the Delta SARS-CoV-2 variant with 28-day hospital mortality between December 2020 and September 2021

Dear Editor,

The spreading of SARS-CoV-2 variants of concern (VOCs) have been associated with a surge of COVID-19 cases and the burden of the healthcare systems worldwide (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants). In response, the Reference center for the Network of COVID-19 Genomic Surveillance in the Canary Islands (Spain) was established in our laboratories by the regional public health system. We tracked the early emergence of the Alpha variant [1] and described the introduction and dynamics of four VOCs in the region from December 2020 to September 2021 [3]. There are major concerns that some VOCs may cause increased disease severity. In this journal, Strålin and colleagues have reported higher rates of hospitalization, severe illness and death among those infected with the Alpha variant (B.1.1.7) compared to non-VOC lineages [5]. Since the Delta (B.1.617.2) variant has been associated with even higher disease severity compared with Alpha (B.1.1.7) [6], here we aimed to retrospectively assess the association between infection by the two major VOCs circulating in the Canary Islands (Spain) archipelago during the period and 28-day mortality among a cohort of hospitalized patients.

The study was conducted at the University Hospital Nuestra Señora de Candelaria (HUNSC) (Santa Cruz de Tenerife, Spain). The institutional review board approved the study (approval CHUNSC_2020_24). As previously described [3], nasopharyngeal swab samples were collected in the Canary Islands archipelago from December 2020 to September 2021. Of the 8224 samples analysed throughout the study period, clinical and patient data were available for only a subset of 5544 samples, out of which 532 samples were collected from hospitalized patients in our center. To exclude samples from patients who attended the hospital for causes unrelated to COVID-19 or who could have had a nosocomial SARS-CoV-2 infection, we included in the analysis only those patients who were hospitalized between one and 21 days from symptoms onset. Sample collection, genome sequencing and lineage assignment are briefly described in the supplementary material and further detailed elsewhere [3]. Logistic and Cox proportional hazard regression models adjusting for patient age, sex, days of hospital stay, and personal history of comorbidities were used to assess whether infection by VOCs in hospitalized patients was associated with 28-day hospital mortality.

Clinical and demographic information of the study population is included in **Table S1**. A total of 423 genome sequences were identified as Alpha (B.1.1.7) or Delta (B.1.617.2 and sublineages). Delta was found in a slightly lower proportion of elderly (>50 years) patients (59.2%) compared with Alpha (65.9%), probably reflecting a larger proportion of vaccinated individuals in that age range when Delta was circulating (May to September 2021) [3]. Despite this, and the higher proportion of vaccination when Delta circulated (50–75% of the population with ≥1 dose between July and September 2021) than when Alpha cir-

Table 1
Association results of SARS-CoV-2 VOCs infections with 28-day mortality among hospitalized patients.

	Logistic regression Odds Ratio (95% CI)	*p*-value	Cox regression Hazard Ratio (95% CI)	*p*-value
Lineage (Delta (B.1.617.2 and sublineages) *vs.* Alpha (B.1.1.7))	2.24 (1.11–4.78)	0.029	2.28 (1.18–4.39)	0.014
Age (years old)	1.06 (1.03–1.09)	4.68×10^{-6}	1.05 (1.03–1.07)	1.20×10^{-6}
Sex (male)	2.45 (1.17–5.40)	0.020	2.03 (1.06–3.88)	0.031
Length of hospital stay (days)	0.98 (0.95–1.01)	0.415	0.93 (0.89–0.97)	2.64×10^{-4}
Comorbidities*	1.65 (0.69–4.21)	0.271	1.55 (0.66–3.60)	0.309

CI, confidence interval.
*Detailed in Table S1.

culated (1–35% of the population with ≥1 dose between January and June 2021) (https://www.sanidad.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/pbiVacunacion.htm), infection by Delta lineages was associated with higher 28-day hospital mortality compared to that of Alpha irrespective of the model (Table 1). Age and male sex were also consistent predictors of higher 28-day hospital mortality.

As a limitation, we did not have access to the vaccination status of the patients. This is mainly due to the lack of a centralized system in Spain where patient vaccination status is stored and to the large proportion of the floating or non-resident population in the islands, such as holidaymakers, nomadic workers, or migrants, who lack health status information in the local databases. We aimed to mitigate the bias caused by this by focusing on hospitalized patients, under the idea that their vaccination level was less relevant for disease severity once the patient was hospitalized. Taken together, hospitalized cases with Delta or any of its sub-lineages were associated with higher mortality compared to those with Alpha, adding to the evidence supporting that Delta was more severe than pre-existent SARS-CoV-2 variants [2,4,6].

Author contributions

CF conceived the idea, the experimental design and supervised the project. LC, JAF, JMLS, HRP, HGC, AIC, RGM, and DGMA conducted the sequencing experiments. JAF, HGC, ODG, and DGMA collected patient data. LC, JMLS, AVF, and CF performed the analysis and interpreted the results. CF obtained the funding. CF and LC drafted the first version of the manuscript and prepared the tables. All authors contributed to manuscript revision and read and approved the submitted version.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.030.

References

1. Alcoba-Florez J., et al. Monitoring the rise of the SARS-CoV-2 lineage B.1.1.7 in Tenerife (Spain) since mid-December 2020. *J Infect* 2021;**82**(6):e1–3 Elsevier. doi:10.1016/j.jinf.2021.04.005.
2. Bager P., et al. Hospitalisation associated with SARS-CoV-2 delta variant in Denmark. *Lancet Infect Dis* 2021;**21**(10):1351 Elsevier. doi:10.1016/S1473-3099(21)00580-6.
[3]. Ciuffreda L., et al. Tracing the trajectories of SARS-CoV-2 variants of concern between December 2020 and September 2021 in the Canary Islands (Spain). *medRxiv* 2022. doi:10.1101/2022.02.26.22271544.
[4]. Freire Rodrigues E., et al. B.1.617.2 SARS-CoV-2 (Delta) variant is associated with increased risk of hospitalization and death compared with B.1.1.7 SARS-CoV-2 (Alpha) variant. *medRxiv* 2022. doi:10.1101/2022.01.21.22268602.
5. Strålin K., et al. Impact of the Alpha VOC on disease severity in SARS-CoV-2-positive adults in Sweden. *J Infect* 2022;**84**(1):e3–5 Elsevier. doi:10.1016/j.jinf.2021.08.043.
6. Twohig K.A., et al. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a cohort study. *Lancet Infect Dis* 2022;**22**(1):35–42 Elsevier. doi:10.1016/S1473-3099(21)00475-8.

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Very low rates of severe COVID-19 in children hospitalised with confirmed SARS-CoV-2 infection in London, England"

We read with interest the publication by Chappell et al. reporting a very low risk of severe COVID in immunosuppressed children in England.[1]

Compared to adults, children and young people (CYP) are more likely to remain asymptomatic or develop mild symptoms when exposed to SARS-CoV-2, and less likely to develop severe disease, be hospitalised, or die of COVID-19.[2] In adults, hospitalisation and case fatality rates due to COVID-19 are regularly used to measure disease severity with new variants and following vaccination.[3] The disconnect between infection and severe disease after successful COVID-19 immunisation programmes, for example, has been a primary driver for many countries to remove mitigations even with the emergence of more transmissible variants.

In CYP, COVID-19 fatalities are rare and the use of COVID-19 hospitalisations as a proxy for disease severity,[4] is questionable because of the high rates of incidental (asymptomatic or mild) infections identified during routine SARS-CoV-2 screening of all CYP presenting to hospital. Two years into the pandemic, there are still limited data on the risk of hospitalisation for severe COVID-19 in CYP. We, therefore, undertook a retrospective case-note review of CYP admitted to a large paediatric hospital in London with PCR-confirmed SARS-CoV-2 infection over 14-month period covering the alpha, delta and omicron variant waves (Fig. 1a and b).

St. George's Hospital is a large teaching hospital providing acute paediatric A&E, general and specialist paediatric, oncological, surgical and neurosurgical, orthopaedic and trauma, NICU and PICU care for children in South London. CYP aged 0–18 years with a SARS-CoV-2 PCR-positive result between 01 December 2020 and 31 January 2022 were identified through the hospital microbiology laboratory database and their electronic medical records reviewed by two independent paediatricians. Case rates for 0–18-year-olds in London were obtained from national community testing data.[5] We excluded children with PIMS-TS, which typically occurs 2–6 weeks after SARS-CoV-2 infection by which time SARS-CoV-2 PCR-tests are usually negative. Confirmed cases were categorised into:

(i) Incidental: admitted for an unrelated illness, but SARS-CoV-2 PCR-positive on routine screening
(ii) Contributory: SARS-CoV-2 potentially contributed to hospitalisation
(iii) Severe COVID-19: SARS-CoV-2 was the primary reason for hospitalisation

During the 14-month period, there were 33,775 CYP A&E attendances and 3593 hospitalisations at St. George's Hospital. Of these, 147 were hospitalised with a positive SARS-CoV-2 PCR-test. The distribution of incidental and contributory cases closely followed community infection rates in London, but hospitalisations for severe COVID-19 occurred mainly during the alpha wave, with very few cases during the delta or omicron waves despite large numbers of community infections in the same age-group.

Incidental infections accounted for 83 (57%) cases and included CYP admitted for surgical (24/83, 29%), neurological (12/83, 14%), trauma (10/83, 12%) and other illnesses (e.g. eating disorders, neonatal jaundice; 37/83,45%). Cases where SARS-CoV-2 likely contributed to hospitalisation (49/147, 33%) had clinical presentations that were typical of other childhood viral illnesses. Most CYP (32/49, 65%) presented with fever and respiratory symptoms, or fever with inadequate oral intake, which warranted admission for observation and/or sepsis screen with antibiotic cover until bacterial cultures returned negative after 48 h; 26% (13/49) in this category were aged <3 months. Other presentations included acute exacerbation of asthma (7/49, 14%), febrile convulsions (4/49, 8%) and other presentations (e.g. diarrhoea and vomiting, feeding difficulties, lethargy; 6/49, 12%). Symptoms were generally mild and only five required supplemental oxygen during their short hospital stay.

Thus, only 15 children (10%) were hospitalised with severe COVID-19 presenting as pneumonitis, mainly during the alpha variant wave (10/15, 67%), and in older CYP (9/15 [60%] were aged 12–18 years) with comorbidities (11/15, including 8 with immunosuppression) (Table 1). Thirteen CYP required supplemental oxygen, seven required intensive care and all recovered.

In our hospital, more than half the CYP hospitalised with a positive SARS-CoV-2 PCR-test had incidental infection. In a further third, SARS-CoV-2 likely contributed to hospitalisation with clinical presentations that were typical of other respiratory viruses that are currently not circulating in England because of the physical distancing measures imposed during various lockdowns.[6] This group included mainly high-risk CYP (young infants, immunocompromised) who typically presented with fever and mild respiratory symptoms but were hospitalised to rule serious underlying infection. Of the few CYP hospitalised with COVID-19 pneumonitis, most were adolescents and nearly all had underlying conditions, especially immunosuppression. They all recovered, consistent with current literature reporting favourable outcomes, including for CYP with comorbidities and immunosuppression.[1,7] Vaccinating children against COVID-19 will further reduce the small risk of severe disease,[8-10] but, since current vaccines provide only limited short-term protection against infection or mild disease,[8,9] the virus will likely continue to contribute indirect hospitalisations, just like other respiratory viruses. In conclusion, unlike adults, hospitalisation with a positive SARS-CoV-2 PCR-test is not a useful marker of severe COVID-19 in children. Our findings demonstrate that, not only do CYP have a very low risk of hospitalisation with SARS-CoV-2 infection, but the vast majority of hospitalised CYP (especially healthy CYP) with confirmed SARS-CoV-2 infection do not have severe COVID-19, irrespective of SARS-CoV-2 variant, while the minority with severe COVID-19 recovered without complications. The very low rate of severe COVID-19 during the omicron variant wave is also reassuring.

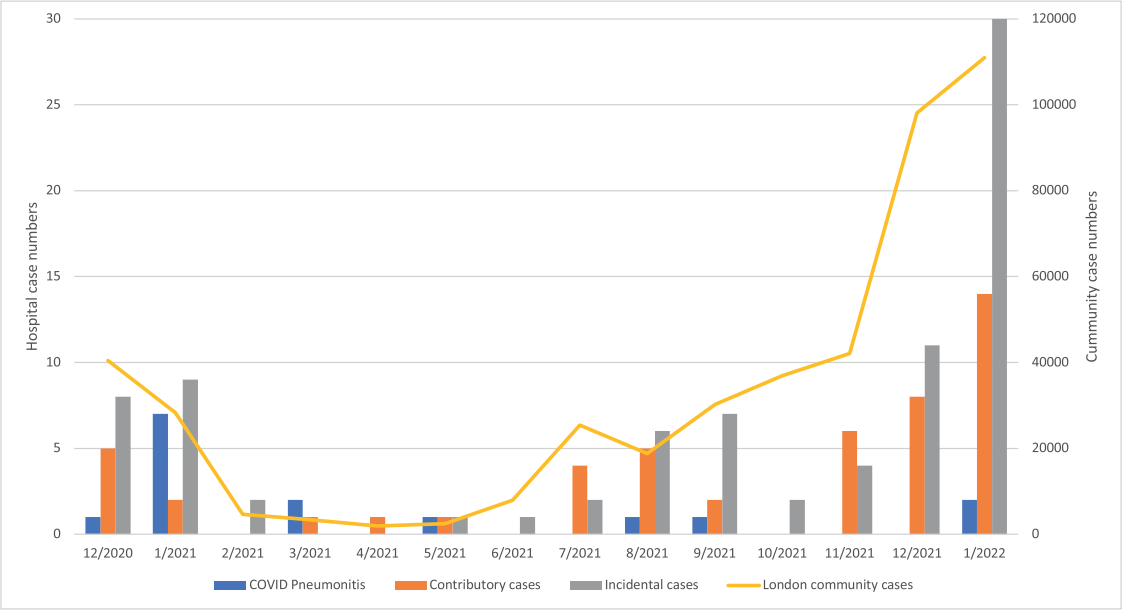

Fig. 1a. Children and young people aged 0–18 years who were admitted to St. George's Hospital, London, with PCR-confirmed SARS-CoV-2 infection and total case numbers for the same age-group in London by month of year during December 2020 to January 2022. The blue bars denote children hospitalised for acute COVID-19, the orange bars denote hospitalisations where SARS-CoV-2 likely contributed to the hospitalisation and the grey bars denote incidental infections in children and young people hospitalised with other medical conditions.

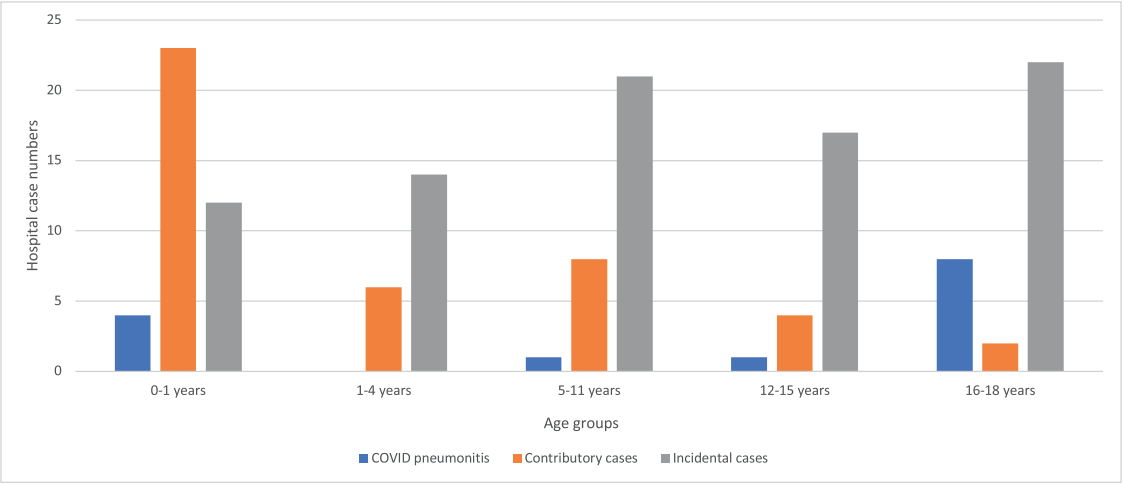

Fig. 1b. Children and young people aged 0–18 years who were admitted to St. George's Hospital, London, with PCR-confirmed SARS-CoV-2 infection by age-group during December 2020 to January 2022. The blue bars denote children hospitalised for acute COVID-19, the orange bars denote hospitalisations where SARS-CoV-2 likely contributed to the hospitalisation and the grey bars denote incidental infections in children and young people hospitalised with other medical conditions.

Declaration of Competing Interest

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Supplementary materials

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References

1. Chappell H., Patel R., Driessens C., et al. Immunocompromised children and young people are at no increased risk of severe COVID-19. *J Infect* 2022;**84**(1):31–9.
2. Castagnoli R., Votto M., Licari A., et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. *JAMA Pediatr* 2020;**174**(9):882–9.
3. Andrews N., Tessier E., Stowe J., et al. Duration of protection against mild and severe disease by Covid-19 vaccines. *N Engl J Med* 2022;**386**(4):340–50.
4. Gurdasani D., Bhatt S., Costello A., et al. Vaccinating adolescents against SARS–CoV-2 in England: a risk-benefit analysis. *J R Soc Med* 2021;**114**(11):513–24.
5. UK Health Security Agency. Official Statistics: National flu and COVID-19 surveillance reports: 2021 to 2022 season. Available at: https://www.gov.uk/government/statistics/national-flu-and-covid-19-surveillance-reports-2021-to-2022-season (accessed 05 February 2022.
6. Taylor A., Whittaker E.. The changing epidemiology of respiratory viruses in children during the COVID-19 pandemic: a Canary in a COVID Time. *Pediatr Infect Dis J* 2022;**41**(2):e46–ee8.

Table 1
Supplement Table. Characteristics, clinical presentation, management and outcomes of children and young people aged 0–18 years with PCR-confirmed SARS-CoV-2 infection who were hospitalised with acute COVID-19 during December 2020 to January 2022.

	Age	Underlying condition	Clinical Presentation	Hospital days	ICU days	Oxygen	Treatment
1.	<1 year	Prematurity	Respiratory distress, pneumonia	3	3	Y	None
2.	<1 year	Prematurity, chronic lung disease	Apnoea, pneumonia	8	6	Y	None
3.	<1 year	Primary immunodeficiency	Chronic diarrhoea, failure to thrive, then pneumonia	8	-	N	None
4.	<1 year	None	Pneumonia	2	-	N	None
5.	5–11 years	Malignancy	Febrile neutropaenia	29	-	Y	Steroids and antivirals
6.	5–11 years	Malignancy	Gram-negative bacterial sepsis, then pneumonia	13	2	Y	None
7.	12–18 years	Malignancy	Gram-negative bacterial sepsis, then pneumonia	10	-	Y	Steroids and antivirals
8.	12–18 years	Malignancy	Febrile neutropenia and pneumonia	10	2	Y	2 admissions: Steroids and antivirals (1st admission), antivirals (2nd admission)
9.	12–18 years	Chronic heart disease	Pneumonia	17	7	Y	Steroids and antivirals
10.	12–18 years	Malignancy	Pneumonia	10	1	Y	Steroids and intravenous immunoglobulin
11.	12–18 years	None	Pneumonia	6	-	Y	Steroids and antivirals
12.	12–18 years	Malignancy	Febrile neutropenia, pneumonia	5	1	Y	Steroids
13.	12–18 years	Primary immunodeficiency	Prolonged fever	10	-	N	Antivirals
14.	12–18 years	None	Fever and dehydration	1	-	N	None
15.	12–18 years	Chronic liver disease	Pneumonia	4	-	Y	Steroids

ICU, intensive care unit.

7. Ward J.L., Harwood R., Smith C., et al. Risk factors for PICU admission and death among children and young people hospitalized with COVID-19 and PIMS-TS in England during the first pandemic year. *Nat Med* 2022;**28**(1):193–200.
8. Powell A.A., et al. Adolescent vaccination with BNT162b2 (Comirnaty, Pfizer-BioNTech) vaccine and effectiveness of the first dose against COVID-19: national test-negative case-control study, England. *MedRxiv* 2021. doi:10.1101/2021121021267408.
9. Prunas O., Weinberger D.M., Pitzer V.E., et al. Waning effectiveness of the BNT162b2 vaccine against infection in adolescents. *medRxiv* 2022. doi:10.1101/2022.01.04.22268776.
10. Olson S.M., Newhams M.M., Halasa N.B., et al. Effectiveness of BNT162b2 vaccine against critical Covid-19 in adolescents. *N Engl J Med* 2022. doi:10.1056/NEJMoa2117995.

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How China responds to Omicron

Dear Editor,

Recently, a letter in your journal predicted that the SARS-CoV-2 Omicron strain will thereby replace the Delta variant as the dominant strain.[1] This prediction has become ever more salient due to the new wave of COVID-19 outbreaks that has occurred in China. This round of epidemic is mainly caused by the Omicron subtype BA.2. Since the day when the first Omicron cases were confirmed and reported to April 3rd 2022, more than 100-thousand people had been infected. The current epidemic in China is unique in its extremely high infection rate as well as the high frequency of its outbreaks over the entire country, which has caused serious challenges in the prevention and control of the epidemic.

Compared with the original and the Delta strains, the replication ability of the BA.2 strain in the lungs is greatly reduced, rendering the virus' pathogenicity greatly weakened.This has resulted in symptoms that are milder than influenza and close to the level of the common cold.[2] The hospitalization rate and ICU rate of infected persons have decreased, and the median period of hospitalization has also decreased.[3] Nevertheless, the BA.2 strain has become extremely infectious—the R0 may be as high as 9. Even with the lower pathogenicity and fatality rate, the mortality rate will increase significantly when a large-scale outbreak occurs, with the death concentrated among the elderly, especially those who have comorbidities and yet to be vaccinated.[4] Recent lessons from Hong

Abbreviations: Omicron, SARS-CoV-2 Omicron variant; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

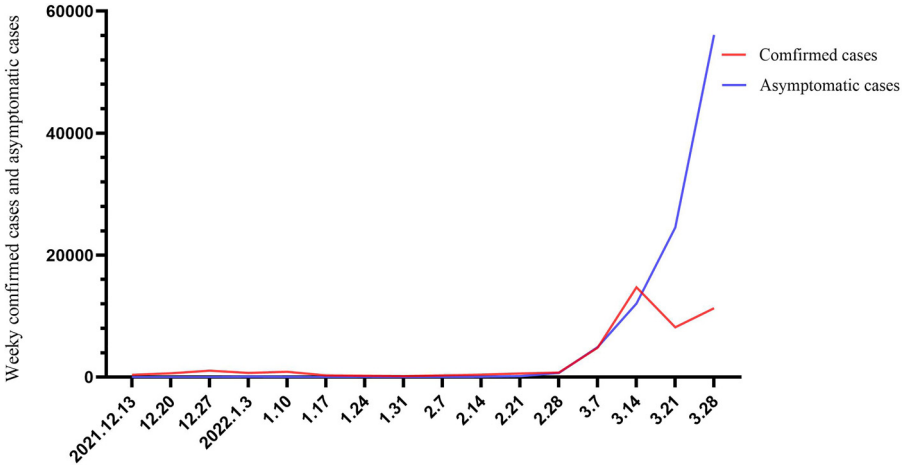

Fig. 1. Weeky comfirmed cases and asymptomatic cases in China from December 13, 2021 to April 3, 2022. Data for all cases are from National Health Commission of the People's Republic of China (http://www.nhc.gov.cn).

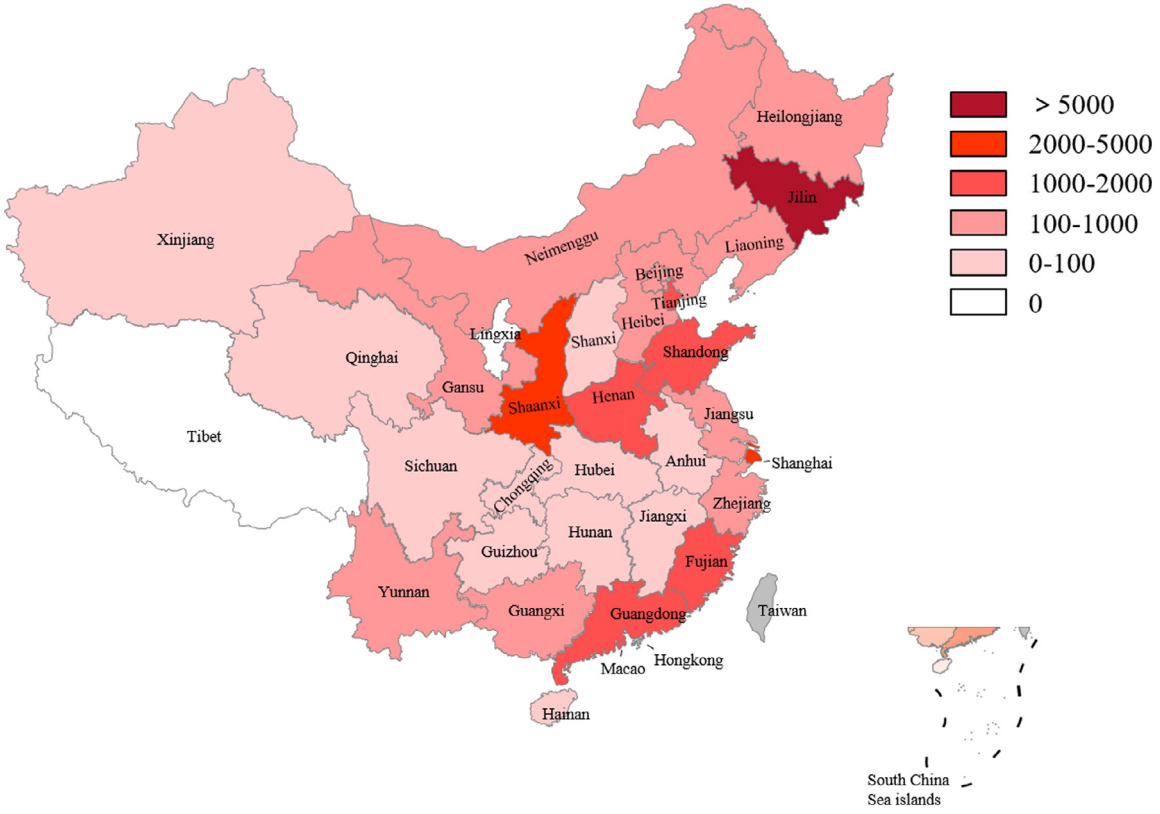

Fig. 2. Geographical distribution of confirmed cases of COVID-19 in China from December 13, 2021 to April 3, 2022, except Macao, Hongkong and Taiwan. Data for all cases are from National Health Commission of the People's Republic of China (http://www.nhc.gov.cn).

Kong exemplify this phenomenon, indicating that improving vaccination rates among the older population should be a high priority[5] (Figs. 1 and 2).

On March 31st, a total of 3.27 billion doses of the novel coronavirus vaccines were purportedly distributed nationwide, with the total number of fully vaccinated people reaching 1.27 billion. This means that around 90.47% of the total population have been vaccinated and 90.63% of people have completed the booster immunization in China. In addition, 223.708 million elderly people over the age of 60 have received the vaccination, 95% of whom have been fully vaccinated. In some provinces, the vaccination rate of elders has reached over 85%.

Learning from the findings of a large number of medical research and clinical practices and considering the dynamics in the domestic and global epidemic situation, the National Health Commission and the State Administration of Traditional Chinese Medicine issued the "Novel Coronavirus Pneumonia Diagnosis and Treatment Plan (Trial Version 9)" to deal with the new outbreak of asymptomatic infected individuals with Omicron variant and a high number of mild cases in China. In conjunction with the aforementioned changes in treatment protocols, several measures have been implemented to deal with Omicron in China in order to more efficiently implement the "Dynamic zero" policy. Firstly, the Paxlovid treatment tablets have been distributed to the frontline, with the hope of reducing hospital admissions and deaths among high-risk individuals.[6] Secondly, in order to further improve the effectiveness of early detection of infected persons, the antigen test kits have been added to the detection protocol so as to reduce

the testing pressure for local laboratories. Lastly, 19 provinces have established mobile cabin hospitals to isolate infected people. Economic centers such as Shenzhen and Shanghai have even implementedd a complete lockdown of the city to stifle the spread of Omicron.

In addition to the several measures have been announced to deal with Omicron, the government should continue implementing the following measures: 1) Studies have shown that a third dose of vaccination could greatly improve the protection rate, thus the government should continue promoting further vaccination in the future in order to maintain the level of community immunity. 2) Epidemic prevention policies should be designed in a way that would not overburden current medical systems whilst ensuring that patients suffering from basic illness, cancer and other diseases would receive the timely treatment and care they deserve. 3) "Dynamic zero" policy should remain in place. The symptoms caused by Omicron infection are mild, hence the public won't be as cautious as they used to be. The government should respond to the concept of coexistence with the virus rationally and put the safety of the people in the first place by educating the public on the potential harm of Omicron strains. 4) Strict mobility policy should be executed to limit the movement of people in medium to high-risk areas for the purpose of preventing the spread of the virus. In summary, as the SARS-CoV-2 continues mutate, the Chinese government should be more vigilant and adjust virus prevention policy dynamically and accordingly to avoid further epidemic of outbreaks while minimizing economic losses. In the near future, when China successfully overcomes Omicron even other variant strains, other countries may learn from China's "Dynamic zero" policy and successfully eliminate SARS-CoV-2 epidemics in the world together.

Declaration of Competing Interest

None.

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References

1. Yadav PD, Sapkal GN, Sahay RR, Potdar VA, Deshpande GR, Patil DY, et al. Substantial immune response in Omicron infected breakthrough and unvaccinated individuals against SARS-CoV-2 variants of concern. *J Infect* 2022.
2. Dimeglio C, Migueres M, Mansuy JM, Saivin S, Miedouge M, Chapuy-Regaud S, et al. Antibody titers and breakthrough infections with Omicron SARS-CoV-2. *J Infect* 2022.
3. He X, Hong W, Pan X, Lu G, Wei X.. SARS-CoV-2 Omicron variant: Characteristics and prevention. *MedComm* 2020:2021.
4. Araf Y, Akter F, Tang YD, Fatemi R, Parvez MSA, Zheng C, et al. Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. *J Med Virol* 2022;**94**(5):1825–32.
5. Cheung PH, Chan CP, Jin DY.. Lessons learned from the fifth wave of COVID-19 in Hong Kong in early 2022. *Emerg Microbes Infect* 2022:1–15.
6. Wang Z, Yang L.. In the age of Omicron variant: Paxlovid raises new hopes of COVID-19 recovery. *J Med Virol* 2022;**94**(5):1766–7.

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High vaccination coverage slows down genetic diversity of SARS-CoV-2

Dear Editor,

A third dose of COVID-19 vaccine has been proved to be necessary to boost antibody level after vaccination for six months [1]. However, the emerging variants of concern (VOC) such as Delta and Omicron tend to weaken the protection of current vaccines [2]. With the observed waning effectiveness of booster shots [3], whether to get additional doses of vaccine in the future remains an open question. A previous study on influenza showed inadequate vaccination might potentially promote genetic diversity of H5N1 virus [4], but the effect of vaccination on SARS-CoV-2 still remains unknown.

In this study, we downloaded nucleotide sequences of spike protein of SARS-CoV-2 from GISAID [5] (https://www.gisaid.org) and classified them by month from Mar. 2020 to Feb. 2022. Daily vaccination rates were collected from Our World in Data [6]. Increases in genetic diversity of SARS-CoV-2 suggested more mutations, which resulted in more potential VOC and infections [7]. Shannon entropy was used to calculate genetic diversity of viruses on each site or the whole sequence [8]. Considering the time interval between the second and third doses and immunity period of current vaccine, an effective vaccination coverage (EVC) was defined by summation of daily vaccination rates of the second and third doses on the last day of per month minus that of the second dose six months before. Time periods of vaccination were divided into different regions by changes in the trends of EVC. To study relationship between genetic diversity of SARS-CoV-2 and vaccination, correlation analysis of Shannon entropy and EVC were performed.

Three representative countries (United States, Israel and Poland) with EVC evidently declined were selected (Fig. 1). We caught a significant negative correlation between EVC from Dec. 2020 to Oct. 2021 and Shannon entropy with one month delayed above period in United States with Pearson $r < -0.8$ (Fig. 2). Though significant correlation was not observed in the other two countries, the Shannon entropy oscillated in limited ranges and never exceeded the last highest point during the start vaccination region. A sharp decline of EVC appeared in United States, leading to a violent fluctuation of Shannon entropy during the decline region on Nov. and Dec. 2021, it is the time the Omicron variant emerged. Similar trend was observed in Israel after Jun. 2021 but with one month delayed. In Poland, a sharp rise of Shannon entropy was observed in the same time when EVC fell into sluggish growth and then decreased during the decline region. Both above processes in Israel and Poland had high negative correlation with Pearson r value < -0.8. With rapid follow-up of booster shots in recovery region and EVC exceeding 50 percent again, the Shannon

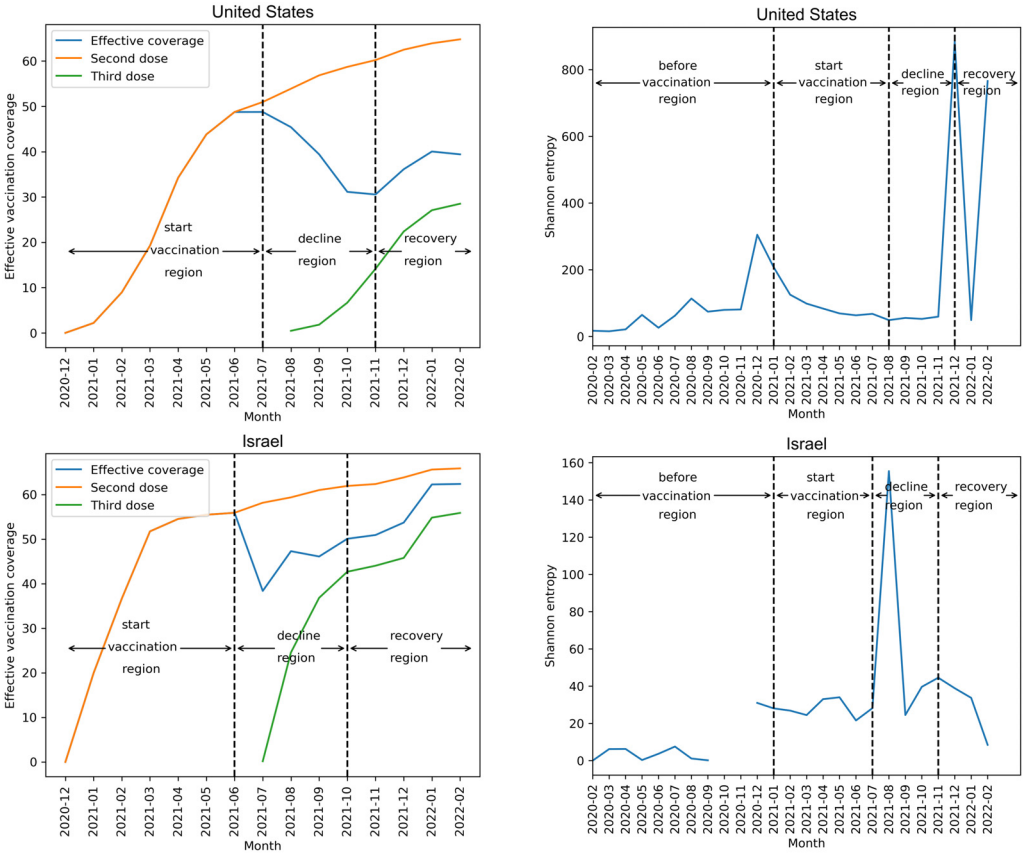

Fig. 1. Tendency of regions with effective vaccine coverage and Shannon entropy in United States, Israel, Poland and Switzerland.

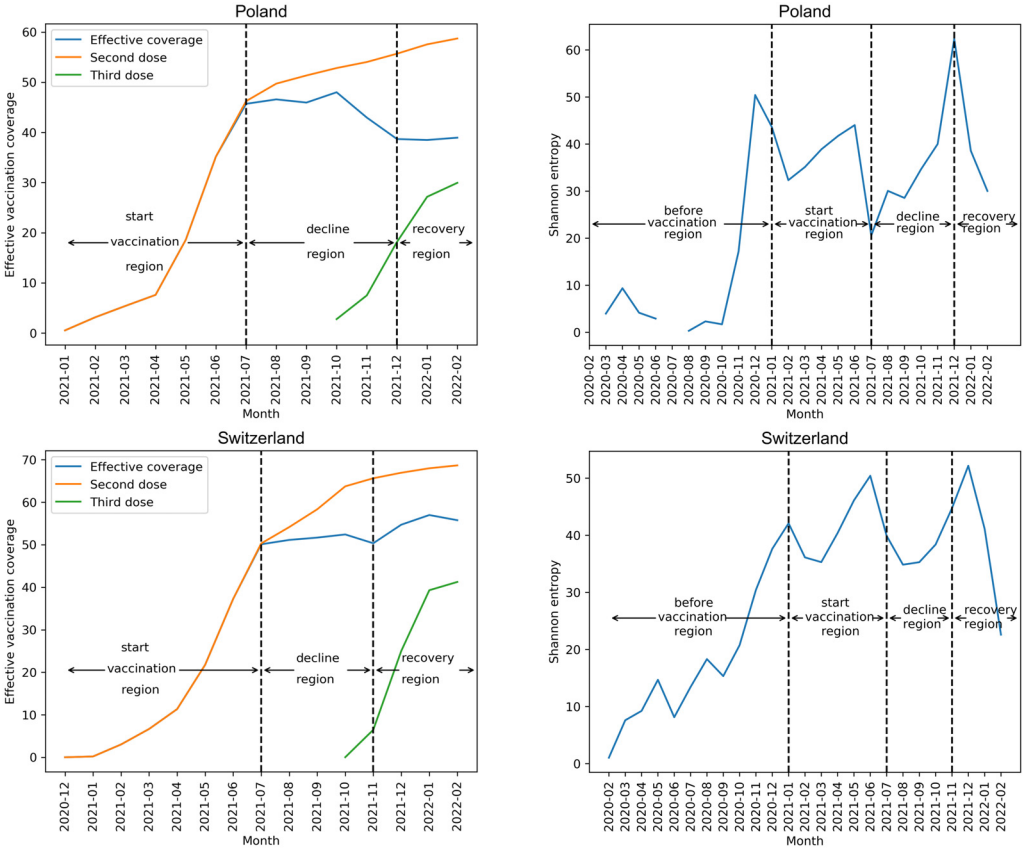

Fig. 1. Continued

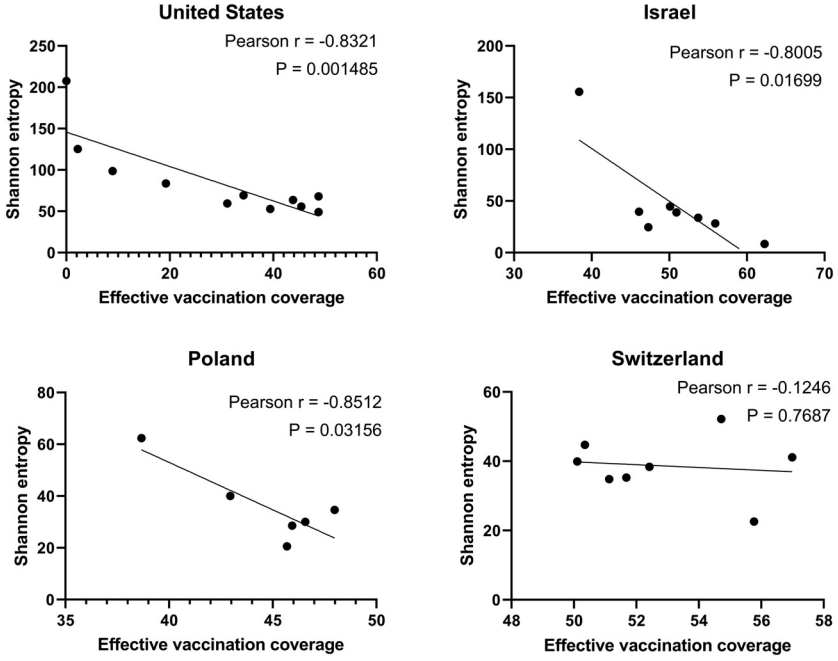

Fig. 2. Correlation analysis of United States by effective vaccination coverage from Dec. 2020 to Oct. 2021 with Shannon entropy one month delayed, Israel from Jun. 2021 to Oct. 2021 with one month delayed, Poland from Jul. 2021 to Dec. 2021 and Switzerland as control from Jul. 2021 to Feb. 2022 in the same period.

entropy was significantly suppressed in Israel. The Shannon entropy evidently declined in recovery region in both United States and Poland, while EVC stabilized at about 40 percent. Shannon entropy rose again immediately in United States in Feb. 2022 with EVC still below 50 percent, however, more data are needed to observe. We supposed that EVC might be not less than 50 percent to have an effect on the genetic diversity of SARS-CoV-2. And another representative country Switzerland was and selected for validation, which had EVC stabilized around 50 percent. Though second dose coverage and EVC diverged from Jul. 2021, Shannon entropy only oscillated in a limited range in decline region in Switzerland and finally declined in recovery region with coverage over 55 percent.

Based on above analysis, we believe continuous EVC over 50 percent may effectively suppress genetic diversity of SARS-CoV-2. Weakening effectiveness of second dose vaccination without booster shot in time may cause a more serious rebound or fluctuation of genetic diversity. It is now generally believed that SARS-CoV-2 might become an endemic virus [9] and evolution of SARS-CoV-2 will continue [10], annual vaccination should be necessary referring to strategy on seasonal influenza. With the potential trend of enhanced immune escape of emerging SARS-CoV-2 variants and weakening of current vaccine [11], our study suggested next booster shot should be carried out before evident weakening of the third dose and strategies of vaccination to quickly reach the rate of EVC over 50 percent should be seriously considered.

Declaration of Competing Interest

None.

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References

1. Tré-Hardy M., Cupaiolo R., Wilmet A., et al. Immunogenicity of mRNA-1273 COVID vaccine after 6 months surveillance in health care workers; a third dose is necessary. *J. Infect.* 2021;**83**:559–64.
2. Accorsi E.K., Britton A., Fleming-Dutra K.E., et al. Association Between 3 Doses of mRNA COVID-19 Vaccine and Symptomatic Infection Caused by the SARS–CoV-2 Omicron and Delta Variants. *JAMA* 2022;**327**:639–51.
3. Levine-Tiefenbrun M., Yelin I., Alapi H., et al. Waning of SARS-CoV-2 booster viral-load reduction effectiveness. *Nat. Commun.* 2022;**13**:1237.
4. Cattoli G., Fusaro A., Monne I., et al. Evidence for differing evolutionary dynamics of A/H5N1 viruses among countries applying or not applying avian influenza vaccination in poultry. *Vaccine* 2011;**29**:9368–75.
5. Elbe S., Buckland-Merrett G.. Data, disease and diplomacy: gISAID's innovative contributionto global health. *Glob. Challenges* 2017;**1**:33–46.
6. Mathieu E., Ritchie H., Ortiz-Ospina E., et al. A global database of COVID-19 vaccinations. *Nat. Human Behav.* 2021.
7. Rahimi A., Mirzazadeh A., Tavakolpour S.. Genetics and genomics of SARS–CoV-2: a review of the literature with the special focus on genetic diversity and SARS-CoV-2 genome detection. *Genomics* 2021;**113**:1221–32.
8. Antuori A., Montoya V., Piñeyro D., et al. Characterization of Acute HCV Infection and Transmission Networks in People Who Currently Inject Drugs in Catalonia: usefulness of Dried Blood Spots. *Hepatology* 2021;**74**:591–606.
9. Lavine J.S., Bjornstad O.N., Antia R.. Immunological characteristics govern the transition of COVID-19 to endemicity. *Science* 2021;**371**:741–5.
10. Telenti A., Arvin A., Corey L., et al. After the pandemic: perspectives on the future trajectory of COVID-19. *Nature* 2021;**596**:495–504.
11. Naranbhai V., Nathan A., Kaseke C., et al. T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. *Cell* 2022.

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Peculiar H3N2 outbreak in São Paulo during summer and emergence of the Omicron variant

Dear Editor,

Influenza virus is associated with considerable morbidity and mortality worldwide. These epidemics occur annually and can be prevented through prophylactic vaccination and mitigated with interventions such as social control measures and specific antiviral use.[1,2] Since the recognition of the widespread community transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), surveillance indicators for influenza have reduced worldwide.[3] The measures implemented for coronavirus disease 2019 (COVID-19), including physical distancing, wearing masks, quarantining, and restricting virus spread/circulation, are also essential to reduce infections caused by influenza and respiratory syncytial viruses; thus, the detection levels of these respiratory viruses became historically low and reduced numbers of influenza viruses were available for WHO characterization than in previous years.[4,5]

Although >65% of the Brazilian population had been fully vaccinated against covid-19 with two doses by the end of 2021, Brazil started the year 2022 with an increase in the number of cases and hospitalizations due to Omicron variant of SARS-CoV-2 and influenza A H3N2 Darwin-like virus also spread of the summer. This study sought to report the outbreak of H3N2 in this context.

This is retrospective, observational study evaluated patients with flu-like symptoms who were admitted to the emergency department of Hospital São Paulo, São Paulo, Brazil, between November 2021 and January 2022. Nasal and nasopharyngeal swabs were collected from the patients and placed in 2 mL of sterile Ringer's lactate solution according to the instructions of the Brazilian Ministry of Health protocol for the management of the influenza A (H1N1) 2009 pandemic. Total RNA was extracted from the samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Subsequently, a one-step quantitative reverse transcription-polymerase chain reaction was performed against the viral M gene, with the human RNase P target as the internal control of the sample quality and normalization factor of the cycle threshold values.

A total of 134 patients with a confirmed diagnosis of influenza A H3N2 were evaluated. The median age was 58.5 years; 24 (17.9%) patients had lung disease, 21 (15.6%) had heart disease, 21 (15.6%) had hematological disease, 9 (6.7%) were pregnant, 9 (6.7%) were aged <5 years, 7 (5.2%) had diabetes, and 6 (4.4%) had nephropathy. Among the patients, 18 (13.4%) required advanced support in the intensive care unit, 8 (5.9%) died, 5 (3.7%) were coinfected with SARS-CoV-2 and one of them died at ICU. A report of previous influenza vaccination was uncommon except for those over 65 years. Fig. 1 shows the relative frequency of the confirmed cases of influenza (H3N2) in the epidemiological weeks starting in November 2021.

Fig. 2 illustrates influenza surveillance performed since the beginning of the pandemic as well as the relative frequency of the cases of COVID-19 and influenza in patients hospitalized with severe acute respiratory syndrome. The graph reflects the ecological niche occupied competitively by the two viruses—a significant decrease in SARS-CoV-2 cases was necessary for the H3N2 outbreak to occur.

This outbreak presents some peculiar features. First, it occurred during the Brazilian summer, an atypical situation when it comes to the seasonal flu virus.[2] Flu circulation during summer is usually minimal because weather conditions are less favorable for the transmission of respiratory droplets and aerosols.[6] Second feature is the low immunity of the population, a few numbers of Influenza B was detected among our hospitalized patients during the beginning of first wave but influenza A/H3N2 infection was not detected among our patients since the pandemic has started.[7,8]. Besides, AH3N2 /Darwin strain was not included in any flu vaccine administered worldwide. Current flu vaccines are updated annually to include strains expected to spread in the upcoming influenza season. Previous H3N2 vaccine strain recommended to the southern hemisphere formulation was the A H3N2/Hong Kong strain but ferret and human antisera react poorly against the Darwin virus in the reference hemagglutination assay.[9] This mismatch between Hong Kong vaccine strain and the circulating AH3N2/Darwin like virus also contribute to increase the susceptibility to infection among population. Finally, previous studies have also reported the occurrence of other respiratory viruses when there is a decline in the number of COVID-19 cases in various parts of the world,[10] further confirming the hypothesis of ecological niche competition among respiratory viruses. Nonetheless influenza outbreaks can be unpredictable and possible co-circulation during future waves of SARS-CoV-2 can occur. Increase immunization and surveillance for respiratory virus infections should be considered part of an essential public health strategy to combat epidemics that are frequent worldwide.

References

1. Iuliano A.D., Roguski K.M., Chang H.H., Muscatello D.J., Palekar R., Tempia S., et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* 2018 Mar 31;**391**(10127):1285–300.
2. Siegers J.Y., Dhanasekaran V., Xie R., Deng Y.-M., Patel S., Ieng V., et al. Genetic and Antigenic Characterization of an Influenza A(H3N2) Outbreak in Cambodia and the Greater Mekong Subregion during the COVID-19 Pandemic, 2020. *J Virol [Internet]* 2021;**95**(24). Nov 23 [cited 2022 Mar 21]Available from https://journals.asm.org/doi/abs/10.1128/JVI.01267-21.
3. Olsen S.J., Azziz-Baumgartner E., Budd A.P., Brammer L., Sullivan S., Pineda R.F., et al. Decreased influenza activity during the COVID-19 pandemic—United States, Australia, Chile, and South Africa, 2020. *Am J Transplant* 2020;**20**(12):3681–5. [Internet]Dec 1 [cited 2022 Mar 21]Available from https://onlinelibrary.wiley.com/doi/full/10.1111/ajt.16381.
4. Sullivan S.G., Carlson S., Cheng A.C., Chilver M.B.N., Dwyer D.E., Irwin M., et al.. *Where Has All the Influenza gone? The impact of COVID-19 On the Circulation of Influenza and Other Respiratory Viruses*, 25, Australia: Eurosurveillance; 2020. March to September[Internet]. 2020 Nov 1 [cited 2022 Mar 21]Available from *https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.47.2001847*.
5. Yeoh D.K., Foley D.A., Minney-Smith C.A., Martin A.C., MacE A.O., Sikazwe C.T., et al. Impact of Coronavirus Disease 2019 Public Health Measures on Detections of Influenza and Respiratory Syncytial Virus in Children During the 2020 Australian Winter. *Clin Infect Dis* 2021;**72**(12):2199–202. [Internet]Jun 15 [cited 2022 Mar 21]Available from https://academic.oup.com/cid/article/72/12/2199/5912591.
6. Tamerius J.D., Shaman J., Alonso W.J., Bloom-Feshbach K., Uejio C.K., Comrie A., et al. Environmental Predictors of Seasonal Influenza Epidemics across Temperate and Tropical Climates. *PLOS Pathog* 2013;**9**(3):e1003194. [Internet][cited 2022 Mar 21]Available from https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003194.
7. Bullard B.L., Weaver E.A.. Strategies Targeting Hemagglutinin as a Universal Influenza Vaccine. *Vaccines* 2021;**9**(3):257. Page 257 [Internet]. 2021 Mar 13 [cited 2022 Mar 21];9Available from https://www.mdpi.com/2076-393X/9/3/257/htm.
8. de Souza Luna L.K., Perosa D.A.H., Conte D.D., Carvalho J.M.A., Alves V.R.G., Cruz J.S., et al. Different patterns of Influenza A and B detected during early stages of COVID-19 in a university hospital in São Paulo, Brazil. *J Infect* 2020 Aug 1;**81**(2):e104–5. [Internet][cited 2022 Mar 28]Available from http://www.journalofinfection.com/article/S0163445320303133/fulltext.
9. WHO. Recommended composition of influenza virus vaccines for use in the 2022 southern hemisphere influenza season [Internet]. 2021

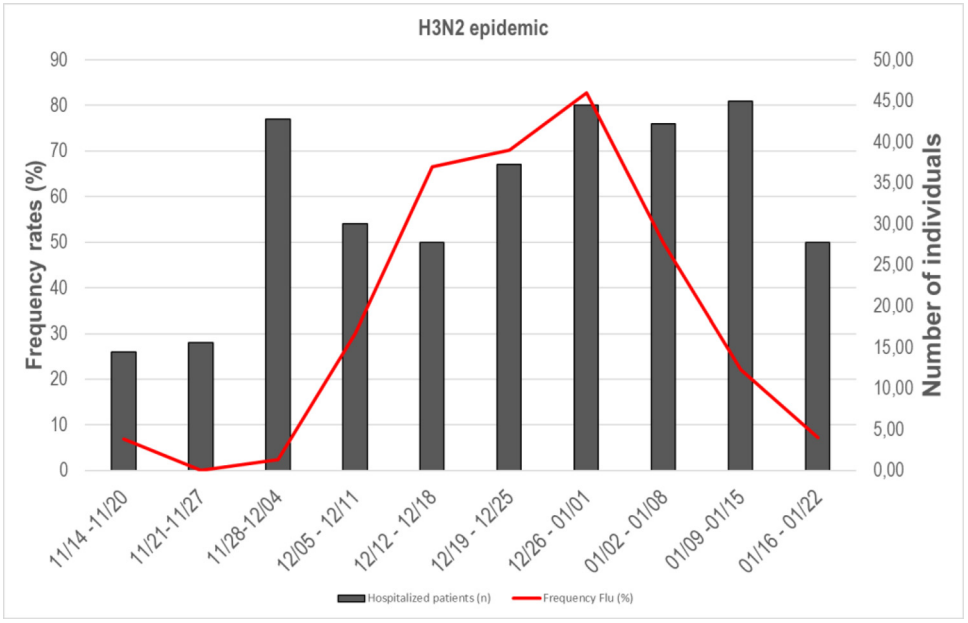

Fig. 1. Relative frequency of the confirmed cases of H3N2 influenza over the epidemiological weeks.

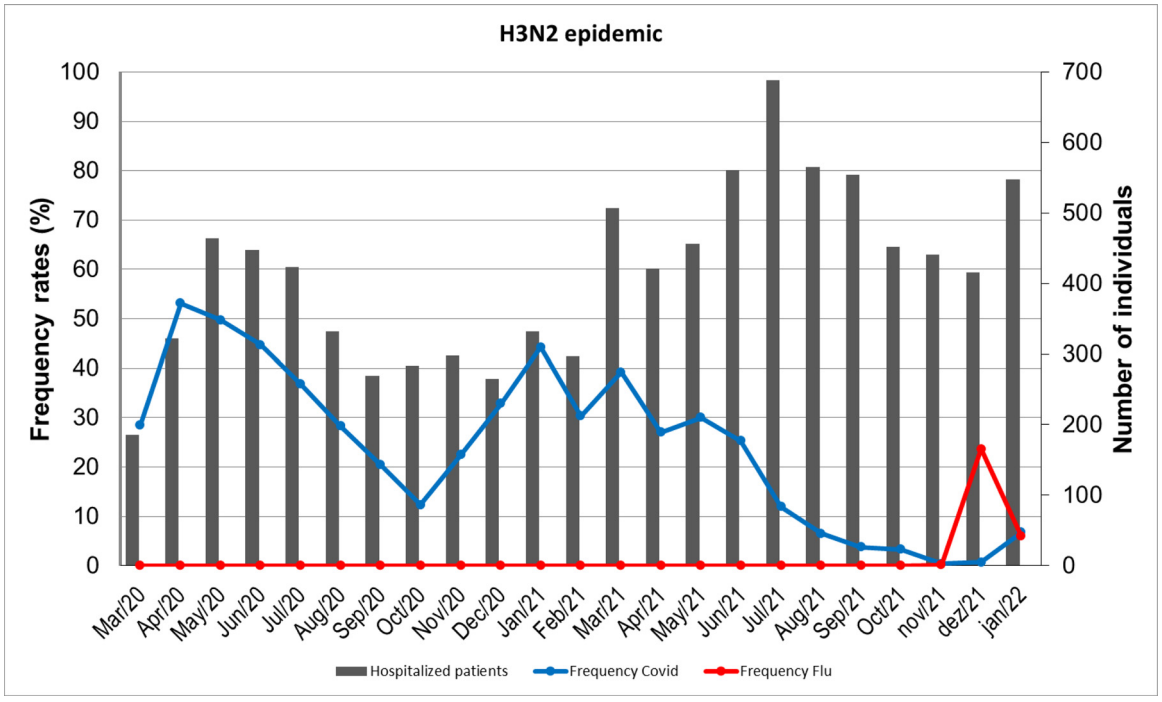

Fig. 2. Relative frequency of COVID-19 and H3N2 influenza during the pandemic in patients hospitalized with severe acute respiratory syndrome.

Sep [cited 2022 Mar 28]. Available from: https://cdn.who.int/media/docs/default-source/influenza/who-influenza-recommendations/vcm-southern-hemisphere-recommendation-2022/202109_recommendation.pdf.
10. Kivit C., Groen K., Jongbloed M., Linssen C., van Loo A., van Gorp E., et al. An off-season outbreak of human metapneumovirus infections after ending of a COVID-19 lockdown. *J Infect* 2022 Feb 3.

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Acute respiratory infections in children, before and after the COVID-19 pandemic, a sentinel study

Dear Editor,

Acute respiratory infection (ARI) is a leading cause of hospital admission of children [1]. The COVID-19 pandemic may pose extra burden to the ARI. China adopted grid governance measures to effectively control the first wave of COVID-19 pandemic [2]. The outbreak of COVID-19 and the strict control measures may shape the pathogen spectrum of ARI [3]. Nationwide prospective surveillance of ARI during a 10-year period in China has been reported [4]. In this study, we investigated the etiological feathures of 440 young patients who were diagonised as acute respiratory infection in a sentinel hospital between an interval before the COVID-19 (2019, Oct-2020, Jan) and an interval after the COVID-19 (2020, May-2021, Jan) in China. All the patients were verified COVID-19 negative. Throat swab samples were collected weekly. The median age of the patients was 6 years, 45.9% were male and 54.1% were female.

Nucleic acids from the throat swab samples were used as template for taqman-based real-time PCR as described previouly [5] . We screened 28 pathogens, including viruses such as human parainfluenza virus 1 (HPIV1), human parainfluenza virus 2 (HPIV2), human parainfluenza virus 3 (HPIV3), human parainfluenza virus4 (HPIV4), influenza A virus (IVA), influenza B virus (IVB), human rhinovirus (HRV), human metapneumovirus (HMPV), human respiratory syncytial virus (RSV), human bocavirus (HboV), human adenovirus (HAdV), human coronavirus 229E (HCoV-229E), human coronavirus NL63 (HCoV-NL63), human coronavirus HKU1 (HcoV-HKU1), human coronavirus OC43 (HcoV-OC43); bacteria such as *Pseudomonas aeruginosa* (PA), *Moraxella catarrhalis* (Mcat), *Mycobacterium tuberculosis* (Mtb), *Legionella pneumophila* (LP), *Group A Streptococcus* (GAS), *Haemophilus influenza* (Hi), *Staphylococcus aureus* (Sa), *Acinetobacter baumannii* (Ab), *Streptococcus pneumonia* (Sp), *Klebsiella.peneumoniae* (Kp), *Escherichia coli* (EC); *and mycoplasma pneumonia* (Mpn) *and chlamydia pneumonia* (Cpn).

In total, 38.6% (170/440) of the patients tested for all the 28 pathogens had at least positive for one pathogen. And 18.1% (80/440) of the patients were exclusively postive for viruses,

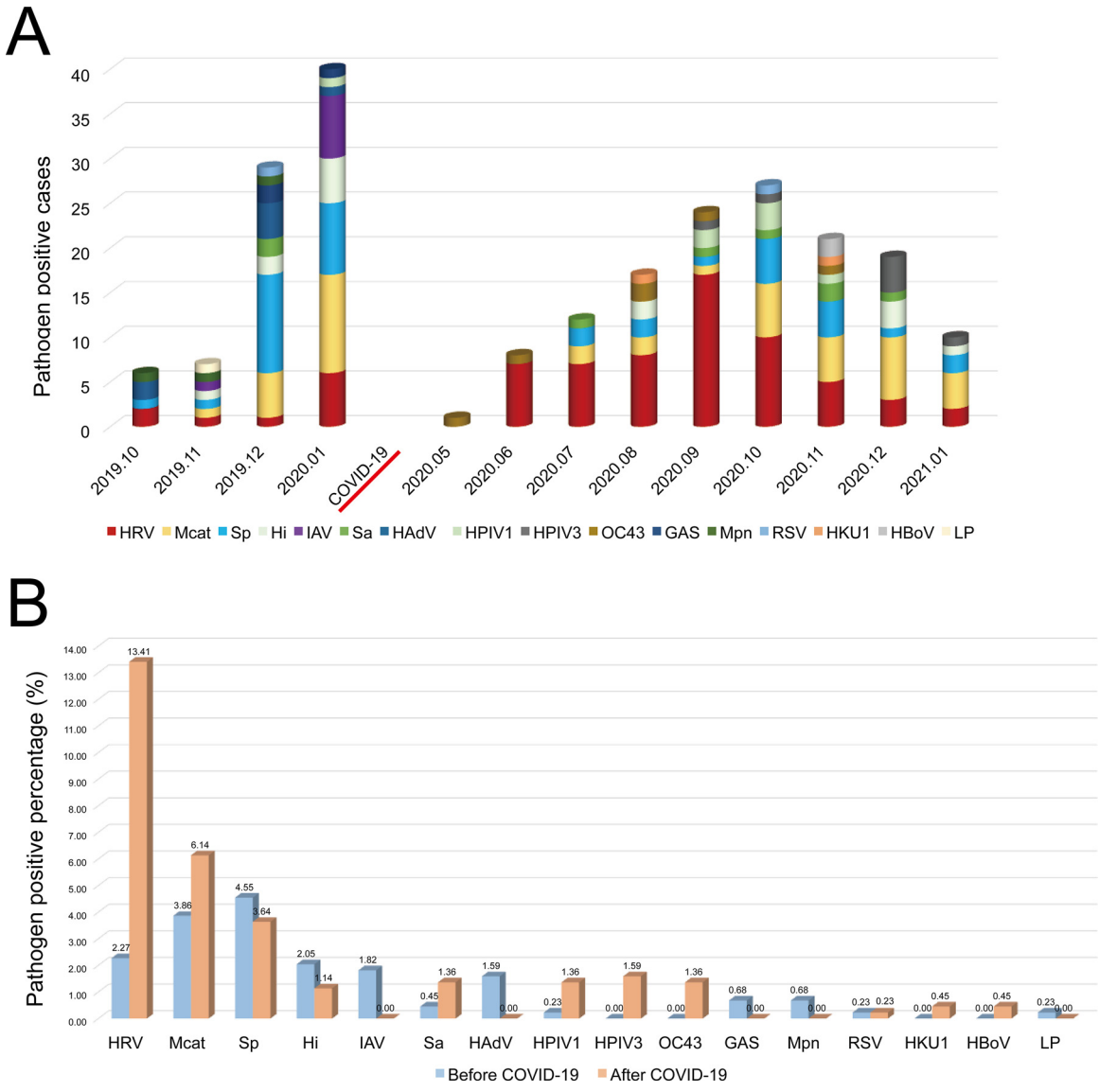

Fig. 1. Pathogen spetrum of acute respiratory infections in children. Throat swab samples were collected from children who were diagonised as acute respiratory infection in the sentinel hospital between an interval before the COVID-19 (2019, Oct-2020, Jan) and an interval after the COVID-19 (2020, May-2021, Jan) in China. (A) Pathogen spetrum tested monthly. (B) Comparsion of the paghogen spectrum betwwen the intervals before and after COVID-19.

Table 1
Positive cases of pathogens in every month.

Date	Patients(Total number)	Virus positivecases	Bacteria positivecases	Other pathogenspositive cases	Viral-bacterial coinfectioncases
2019.10	21	4 (19.05)	1 (4.17)	1 (4.17)	0 (0.00)
2019.11	32	2 (6.25)	4 (12.50)	1 (3.13)	1 (3.13)
2019.12	100	6 (6.00)	16 (16.00)	1 (1.00)	0 (0.00)
2020.01	47	14 (29.79)	20 (42.55)	0 (0.00)	6 (12.77)
COVID-19					
2020.05	2	1 (50)	0 (0.00)	0 (0.00)	0 (0.00)
2020.06	8	8 (100)	0 (0.00)	0 (0.00)	0 (0.00)
2020.07	17	7 (41.18)	5 (29.41)	0 (0.00)	3 (17.65)
2020.08	40	11 (27.50)	4 (10.00)	0 (0.00)	1 (2.50)
2020.09	38	21 (55.26)	2 (5.26)	0 (0.00)	2 (5.26)
2020.10	65	16 (24.62)	10 (15.38)	0 (0.00)	4 (6.15)
2020.11	24	12 (50.00)	12 (50.00)	0 (0.00)	8 (33.33)
2020.12	24	3 (12.50)	6 (25.00)	0 (0.00)	1 (4.17)
2021.01	22	3 (13.64)	6 (27.27)	0 (0.00)	1 (4.55)

13.4% (59/440) of the patients were exclusively postive for bacteria, 6.2% (27/440) of the patients were co-infected with at least one virus and bacterium. Co-infection pattern included HRV/Mcat (5/440), HRV/Hi (3/440), HRV/ Sp (2/440), HRV/Sa (2/440), HPIV3/Mcat (2/440), HRV/IVA/Sp (1/440), IAV/Mcat (1/440), IAV/Sp (1/440), IAV/Sp/Hi (1/440), HPIV1/Sp (1/440), HPIV1/Sp/Sa (1/440), HboV/Mcat (1/440), OC43/Mcat (1/440) and HKU1/Sp (1/440). Overall, there were 15.68% (69/440) patients were postive for HRV, 10% (44) were positive for *M. Catarrhalis*, 8.64% (38/440) were positive for *S. Pneumonia*, 3.18% (14/440) were positive for *H. Influenza, 1.82% (8/440)* were positive for IAV, *1.82% (8/440)* were positive for *S. Aureus, 1.59% (7/440)* were positive for HadV, *1.59% (7/440)* were positive for HPIV1, *1.59% (7/440)* were positive for HPIV3, *1.36% (6/440)* were positive for OC43, 0.68% (3/440) were positive for GAS, 0.68% (3/440) were positive for *M. Pneumonia, 0.45% (2/440)* were positive for RSV, *0.45% (2/440)* were positive for HcoV-HKU1, *0.45% (2/440)* were positive for HboV and 0.23% (1/440) was postive for *L. Pneumophila.*

We analyzed the pathogen spectrum before and COVID-19 pandemic and after the COVID-19 pandemic in every month (Table 1). Before the COVID-19 pandemic (2019, Oct-2020, Jan), there were positive cases for *S. Pneumonia, M. Catarrhalis,* HRV, *H. Influenza, IVA, HadV, GAS, M. Pneumonia, S. Aureus, RSV and L. Pneumophila* (Fig. 1A and 1B). After the COVID-19 pandemic (2020, May-2021, Jan), there were positive cases for HRV, *M. Catarrhalis, S. Pneumonia, H. Influenza, S. Aureus,* HPIV1, HPIV3, OC43, HKU1, HBoV and RSV (Fig. 1A and 1B). Strikingly, after the COVID-19 pandemic, no IVA and HAdV cases were detected and there were increasing cases for HRV, HPIV1, HPIV3 and OC43 (Fig. 1A and 1B), which may suggest that the COVID-19 pandemic shape the pathogen spectrum. The decrease of the seasonal IVA cases is likely due to the COVID-19 lockdown in China and tightly controlled transportation. Although containment strategies were taken in China even after the COVID-19 [6], there were still high rate of HRV infection detected in our study (Fig. 1B).

In summary, we carried out an extensive pathogen screening for acute respiratory infection in children in a sentinel hospital before and after COVID-19 in China. The data suggest that the COVID-19 pandemic shape the pathogen spectrum. And care should sitll be taken for infections of HRV, HPIV and OC43 even under the containment strategies.

Ethics statements

This study was approved by the ethics committee of shool of basic medical sciences, Shanghai medical college under the study number 2018-C010 and the ethics committee of Shanghai Xuhui Central Hospital under the study number IEC-033–02.0-AF02. The procedures were carried out in accordance with approved guidelines. Informed consent was obtained from the subjects.

Authors' contributions

Yi Z conceived the manuscript; YY, HY, CL and SZ collected clinical samples; SW performed experiments; FL and Yuan Z provided resources; SW and Yi Z analyzed date, Yi Z wrote the manuscript. SW and YY contributed equally.

Declaration of competing interest

The authors declare no conflicts of interest.

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References

1. GBD. Mortality, morbidity, and hospitalisations due to influenza lower respiratory tract infections, 2017: an analysis for the Global Burden of Disease Study 2017. Lancet Respir. Med 2019; 7: 69–89. doi:10.1016/S2213-2600(18)30496-X.
2. Wei Yujun, Ye Zhonghua, Cui Meng, Wei Xiaokun. COVID-19 prevention and control in China: grid governance. *J Public Health* 2021;**43**:76–81. doi:10.1093/pubmed/fdaa175.
3. Diesner-Treiber 1 Susanne C., Voitl 1 Peter, Voitl Julian J.M., Langer Klara, Kuzio Ulrike, Riepl Angela, Patel Pia. Alexandra Mühl-Riegler and Bernhard Mühl.Respiratory Infections in Children During a Covid-19 Pandemic Winter. *Front. Pediatr* 2021;**9**:740785. doi:10.3389/fped.2021.740785.
4. Li Z.J., Zhang H.Y., Ren L.L., Lu Q.B., Ren X., Zhang C.H., Wang Y.F., Lin S.H., Zhang X.A., Li J., Zhao S.W., Yi Z.G., Chen X., Yang Z.S., Meng L., Wang X.H., Liu Y.L., Wang X., Cui A.L., Lai S.J., Jiang T., Yuan Y., Shi L.S., Liu M.Y., Zhu Y.L., Zhang A.R., Zhang Z.J., Yang Y., Ward M.P., Feng L.Z., Jing H.Q., Huang L.Y., Xu W.B., Chen Y., Wu J.G., Yuan Z.H., Li M.F., Wang Y., Wang L.P., Fang L.Q., Liu W., Hay S.I., Gao G.F.. Yang WZ; Chinese Centers for Disease Control and Prevention (CDC) Etiology of Respiratory Infection Surveillance Study Team. Etiological and epidemiological features of acute respiratory infections in China. *Nat Commun* 2021;**12**:5026. doi:10.1038/s41467-021-25120-6.
5. Song W., Jia X., Zhang X., Ling Y., Yi Z.. Co-infection in COVID-19, a cohort study. *J Infect* 2021;**82**:440–2. doi:10.1016/j.jinf.2020.10.006.
6. Lai Shengjie, Ruktanonchai Nick W., Zhou Liangcai, Prosper Olivia, Luo6 Wei, Floyd Jessica R., Wesolowski Amy, Santillana Mauricio, Zhang Chi, Du Xiangjun, Yu Hongjie, Tatem Andrew J.. Effect of non-pharmaceutical interventions to contain COVID-19 in China. *Nature* 2020;**585**:410–13. doi:10.1038/s41586-020-2293-x.

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FilmArray Meningitis/Encephalitis (M/E) false-negative for cryptococcal meningitis

Dear Editor,

We read with great interest the article by González-Donapetry et al. on a case of false-negative BioFire® FilmArray® Meningitis/Encephalitis assay (FilmArray® ME panel, BioFire Diagnostics, bioMe ŕieux, Salt Lake City, UT, USA) result for *Neisseria meningitidis* and investigating how they managed to overcome the situation.[1] False-negative cases of bacterial meningitis in FilmArray® ME panel are rarely described in the literature.[1] However, several published case reports have described false-negative FilmArray® ME panel results in patients with cryptococcal meningitis or meningoencephalitis.[2-5]

A 41-year-old man with a history of diabetes mellitus and hypertension, diagnosed since two years ago. without regular medication control, was a carrier of hepatitis B virus. He initially presented with acute onset of dizziness and headache. The symptoms did not improve, and severe dizziness, slurred speech, and mildly blurred vision with unsteady gait developed 4 days later. The patient visited our neurology outpatient clinic for assistance. Neurological examination revealed right dysmetria on the finger-nose-finger test, wide-based gait, and poor rapid alternating movement. Brain magnetic resonance imaging revealed no significant arterial stenosis, occlusion, or acute infarction. He was admitted to the neurology ward under the suspicion of cerebellar stroke. He tested negative for human immunodeficiency virus (HIV) (HIV Ag/Ab Combo, Abbott GmbH, Wiesbaden, Germany). Examination of the cerebrospinal fluid (CSF) obtained on the first hospitalization day revealed negative India ink staining but a high cryptococcal antigen titer of >1,280X (Cryptococcal antigen lateral flow assay, CrAg LFA, IMMY, Norman, OK, USA). The cryptococcal antigen test of the blood sample obtained on the same day was also positive (>2560 ×). However, *Cryptococcus neoformans/gattii* was not detected by the FilmArray® ME panel.

A fungal culture of the CSF sample (approximately 0.2 mL) was grown on Sabouraud dextrose agar (BIOSTAR™, New Taipei City, Taiwan) after incubation for 4 days. The isolate was identified as *C. gattii* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Biotyper; Bruker Daltonics GmbH, Bremen, Germany). The concentration of *C. gattii*in the CSF sample was estimated to be 5 colony-forming units (CFUs) /mL.

To investigate the false-negative results of the FilmArray® ME assay, the growth of *C. gattii* was serially diluted in brain heart infusion broth (BHI, BIOSTAR™, New Taipei City, Taiwan), and the actual concentrations of live organisms in five dilutions (A to E) were 220, 120, 100, 70, and 47 CFU/mL, respectively. *C. neoformans/gattii* was detected in A–D, but not in E, using the FilmArray® ME assay.

Previous reports revealed that the FilmArray® ME panel had a sensitivity and specificity of 96.4% and 99.6% compared with culture, and 83.8% and 99.9% compared to CSF cryptococcal antigen testing, respectively.[2,5] A negative predicted value of 99.9% for the FilmArray® ME panel compared with culture was also reported.[2,5] The majority of false-negative cases have occurred among non-HIV-infected patients with low CSF cryptococcal antigen titers.[2] Our patient did not have HIV infection, however, had high titers of cryptococcal antigen in both CSF blood specimens.

The false-negative results from the FilmArray® ME panel assay from the CSF specimens of patients with microbiologically documented cryptococcal meningitis might also be due to the low fungal load in the CSF specimen, which was lower than the limit of detection (LOD) of the assay. In a cohort of HIV-positive patients with meningitis in Uganda, the FilmArray® ME panel showed 96% sensitivity for cryptococcal detection in CSF samples with the presence of Cryptococcus organisms ≥100 CFU/mL (as determined by quantitative fungal culture).[5] In this study, a concentration of 70 CFU/mL of *C. gattii* in the BHI broth was also detected using FilmArray® ME assay.

In conclusion, cryptococcal meningitis remains an important cause of morbidity and mortality in immunocompromised patients. Laboratory diagnostics for cryptococcal meningitis include antigen detection, India ink staining, and fungal cultures. Detection of *Cryptococcus* species by FilmArray® ME panel appears to rely on the burden of the organisms present. The potential utility of using negative FilmArray® ME panel test results to exclude patients with cryptococcal meningitis requires further investigation.

Ethical approval information

Not required.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

1. González-Donapetry P., García-Rodríguez J., Cendejas-Bueno E. A case of a FilmArray® ME false negative in meningococcal meningitis. *J Infect* 2019;**79**(3):277–87.
2. Van T.T., Kim T.H., Butler-Wu S.M.. Evaluation of the Biofire FilmArray meningitis/encephalitis assay for the detection of *Cryptococcus neoformans/gattii*. *Clin Microbiol Infect* 2020;**26**(10):1375–9.
3. Lee S.H., Chen S.Y., Chien J.Y., Lee T.F., Chen J.M., Hsueh P.R.. Usefulness of the FilmArray meningitis/encephalitis (M/E) panel for the diagnosis of infectious meningitis and encephalitis in Taiwan. *J Microbiol Immunol Infect* 2019;**52**(5):760–8.
4. Lewis P.O., Lanier C.G., Patel P.D., Krolikowski W.D., Krolikowski M.A.. False negative diagnostic errors with polymerase chain reaction for the detection of cryptococcal meningoencephalitis. *Med Mycol* 2020;**58**(3):408–10.
5. Rhein J., Bahr N.C., Hemmert A.C., Cloud J.L., Bellamkonda S., Oswald C., et al. Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagn Microbiol Infect Dis* 2016;**84**(3):268–73.

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Nosocomial septicemia in COVID-19 nosocomial *K. pneumoniae, A. baumannii*, and *Elizabethkingia meningoseptica* septicemia in a patient of COVID-19

To the Editor:

We read with interest the paper by Kwok and colleagues to highlight the antibiotic overuse among hospitalized coronavirus disease 2019 (COVID-19) cases[1]. Although the reported antibiotic prescription rate (29.1%) was lower than previously reported[2], it still remarkably exceeded that of the confirmed bacterial co-infections (1.8%). However, for patients who develop severe hypoxemia, immune modulation with corticosteroids and anti-IL6 are suggested to limit the collateral damage of the cytokine storm[3]. Aggressive supportive care such as central venous catheterization, mechanical ventilation, and extracorporeal membrane oxygenation may be needed for those with respiratory failure and hemodynamic instability. These invasive procedures and immune modulators render these patients vulnerable to nosocomial infections, negatively impacting treatment outcomes. It could be a dilemma whether antibiotics should be used for those with deteriorating multiorgan dysfunctions. Herein, we reported a patient of COVID-19 associated acute hypoxemic respiratory failure who was initially treated with broad-spectrum antibiotics, corticosteroids, tocilizumab, and remdesivir and was successfully liberated from mechanical ventilation. Yet, bacteremia and osteomyelitis of multidrug-resistant nosocomial pathogens ensued, leading to a protracted course of hospitalization.

A 65-year-old man visited the emergency room due to productive cough, lightheadedness, weakness, and shortness of breath for one week during an outbreak of domestic COVID-19. The patient was a teacher of physical education who was in his usual state of health before this episode. He had a past medical history of alcoholism with alcoholic liver cirrhosis and was inconsistently followed up.

He was 176 cm tall and weighed 70 kg. He was febrile, tachypneic, and cyanotic (saturation of peripheral oxygen, SpO_2 87%). His laboratory data showed abnormal liver function tests (aspartate aminotransferase 119 U/l, alanine aminotransferase 89 U/l, and total bilirubin 2.4 mg/dl), elevated infection parameters (C-reactive protein 3.1 mg/dl, procalcitonin 1.07 ng/ml, D-dimer 1.02 mcg/ml and ferritin 4298 ng/ml), and lactic acidosis (pH 7.308, HCO_3 19.1 mmol/l, lactate 8.7 mmol/l). High hemoglobin A1c (9.0%) was noted, but he was previously unaware of a diagnosis of diabetes mellitus. The SARS-CoV-2 reverse transcriptase-polymerase chain reaction was positive. The chest X-ray showed bilateral ground-glass opacities.

After being admitted, he was empirically treated with intravenous ertapenem and levofloxacin (Table. 1). Dexamethasone and remdesivir therapy were started on the first and third days. On the third day, one dose of tocilizumab 600 mg was given alongside. Enoxaparin 40 mg QD was prescribed to prevent thromboembolism.

On the 6th day of admission, with the support of an O_2 mask using a fraction of inspiration oxygen (FiO_2) of 0.6, the partial pressure of oxygen (PaO_2) of arterial blood was 102.1 mmHg (PaO_2/FiO_2 = 170 mmHg). He fainted while briefly leaving the oxygen support, attempting to go to the toilet. Mechanical ventilation was initiated through an endotracheal tube.

Antibiotics were altered to doripenem, levofloxacin, and teicoplanin to cover suspected nosocomial infection. He improved and was liberated from mechanical ventilation three days later. The sputum culture grew normal flora and *Candida albicans,* and the antibiotics were discontinued. However, he became icteric and exhausted on the 19th day. Although he showed no fever or chillness, meropenem and fluconazole were used empirically. The blood culture yielded carbapenem-resistance *Klebsiella pneumoniae, Acinetobacter baumannii, and Elizabethkingia meningoseptica*. The antibiotics were changed to cefoperazone/sulbactam and tigecycline. Yet, spiking fever developed on the 23rd day and jaundice progressed (Total bilirubin 8.47 mg/dl). The antimicrobials were altered to ceftazidime/avibactam and amikacin. Later, the blood culture yielded *Elizabethkingia meningoseptica*. The antibiotics were changed to piperacillin/tazobactam and tigecycline. Jaundice and fever subsided, but the *Elizabethkingia meningoseptica* bacteremia persisted until the 31st day of admission. The antibiotics continued until two weeks after blood cultures converted to negative results.

He kept complaining about lower back pain, and abnormal alkaline phosphatase levels ranging from 110 to 220 U/l persisted. It raised the doubt of unresolved infection foci. A spine MRI revealed an abnormal enhancement in T2-weighted images, suggestive of osteomyelitis, at L3–4 endplates (Fig. 1). Intravenous cefoperazone/sulbactam and tigecycline were administered. Subsequent blood cultures yielded no positive results. After a 14-day course of intravenous antibiotics, oral minocycline and sulfamethoxazole/trimethoprim were used, and the patient was discharged. The oral antibiotics were continued for four months in the outpatient department until the erythrocyte sedimentation rate declined to be within normal limits.

As pointed out by Kwok et al., bacterial co-infection is rare among hospitalized COVID-19 patients[1], and antibiotics should not be prescribed as a routine upon admission. Imprudent use of antibiotics predisposes the patients to multi-resistant nosocomial infections, especially for those with comorbid conditions, advanced age, using immunosuppressants, indwelling invasive devices, and prolonged hospitalizations[4]. On the other hand, delay in adequate antibiotic coverage for patients with sepsis due to bacterial etiologies leads to catastrophic organ dysfunctions and increases the risk of mortality. Procalcitonin, widely used as an indicator for bacterial

Abbreviations: MRI, magnetic resonance imaging; MV, mechanical ventilation.

Table 1
Summary of the admission course, antimicrobial agents, and culture results.

Time (day)	Event	Antibiotics	Culture
−7	Cough, shortness of breath		
1	Admission, dexamethasone given	Ertapenem, levofloxacin	Sputum: normal flora; Blood: negative
3	Tocilizumab and remdesivir used	Ertapenem, levofloxacin	
6	Mechanical ventilation (MV)	Doripenem, levofloxacin, teicoplanin	Sputum: normal flora and *Candida albicans*; Blood: negative
9	Liberation from MV		
19	Jaundice, sepsis	Meropenem, fluconazole	Blood: *A. baumannii, K. pneumoniae, Elizabethkingia meningoseptica*
22		Cefoperazone/sulbactam, tigecycline	
23	Spiking fever jaundice progressed	Ceftazidime/avibactam, amikacin	Blood: *E. meningoseptica*
26		Piperacillin/tazobactam, tigecycline	Blood: *E. meningoseptica*
31			Blood: *E. meningoseptica*
44	Spine MRI: osteomyelitis	Cefoperazone/sulbactam, tigecycline	Blood: negative
58	Discharge	Minocycline, sulfamethoxazole/trimethoprim	Blood: negative

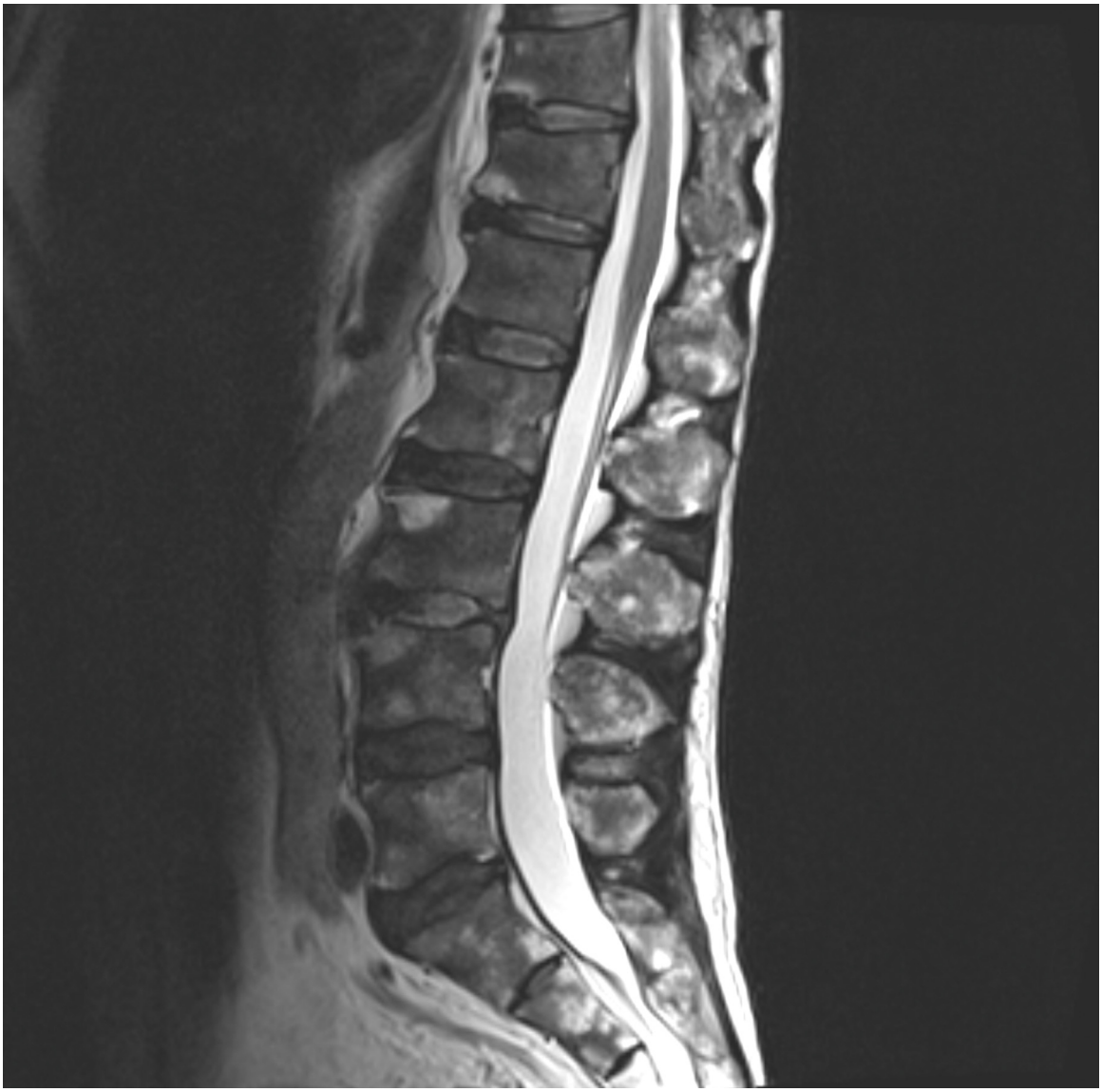

Fig. 1. Contrast enhancement at L3–4 endplate in T2-weighted images of the spine magnetic resonance imaging.

septicemia, also increases in a severe COIVD-19 cytokine storm[5]. As illustrated in the present case, the usefulness of procalcitonin to guide antibiotics therapy was eclipsed in patients with severe COVID-19.

Multiple factors contributed to the increased risk of secondary infection in severe COVID-19. Patients with underlying medical conditions are more likely to progress to severe disease. Suppression of type 1-interferon production by the SARS-CoV2 infection compromises macrophage recruitment and phagocytic function, creating a positive environment for secondary bacterial infections[6, 7]. Contamination of these multidrug-resistant *K. pneumoniae, A. baumannii*, and *Elizabethkingia meningoseptica* frequently found in the environment of intensive care units could be translocated to patients' mucosa or skin surface during daily care[8]. The

use of broad-spectrum antibiotics, especially the carbapenems, decreases the diversity and shifts the equilibrium of microbial flora, favoring these resistant strains. The combination of underlying comorbidities, immune dysfunction, immunosuppressive therapy, and invasive procedures breaching the natural skin and mucosal barriers makes patients of severe COVID-19 prone to secondary nosocomial infections.

Elizabethkingia meningoseptica septicemia and osteomyelitis, although uncommon, is highly resistant to antibiotics and carries a high risk of mortality. Judicious use of antibiotics is suggested to avoid the expansion of multi-drug resistant pathogens, and agile microbiological study is advised if clinical clues of sepsis arise.

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Ethical statement

The Joint Institutional Review Board of Taipei Medical University approved this report (N202203030). The consent for publication was obtained from the patient.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Kwok K.O., Wei W.I., Ma B.H.M., Ip M., Cheung H., Hui E., et al. Antibiotic use among COVID-19 patients in Hong Kong. *J Infect* 2022:20220217 2022 Feb 17PubMed PMID: 35183606. Pubmed Central PMCID: PMC8851745. Epub, In press. doi:10.1016/j.jinf.2022.02.014.
2. Langford B.J., So M., Raybardhan S., Leung V., Soucy J.R., Westwood D., et al. Antibiotic prescribing in patients with COVID-19–Rapid review and meta-analysis. *Clin Microbiol Infect* 2021;**27**(4):520–31 AprPubMed PMID: 33418017. Pubmed Central PMCID: PMC7785281. Epub 20210105.
3. Batista C.M., Foti L. Anti-SARS-CoV-2 and anti-cytokine storm neutralizing antibody therapies against COVID-19–Update, challenges, and perspectives. *Int Immunopharmacol* 2021;**99**:108036 Aug 3PubMed PMID: 34371330. Epub 2021/08/10.
4. Landoas A., Cazzorla F., Gallouche M., Larrat S., Nemoz B., Giner C., et al. SARS–CoV-2 nosocomial infection acquired in a French university hospital during the 1st wave of the COVID-19 pandemic, a prospective study. *Antimicrob Resist Infect Control* 2021;**10**(1):114 Aug 5PubMed PMID: 34353356. Pubmed Central PMCID: PMC8339707. Epub 2021/08/07.
5. Hu R., Han C., Pei S., Yin M., Chen X. Procalcitonin levels in COVID-19 patients. *Int J Antimicrob Agents* 2020;**56**(2):106051 AugPubMed PMID: 32534186. Pubmed Central PMCID: PMC7286278. Epub 20200610.
6. Kalfaoglu B., Almeida-Santos J., Tye C.A., Satou Y., Ono M. T-cell dysregulation in COVID-19. *Biochem Biophys Res Commun* 2021;**538**:204–10 Jan 29PubMed PMID: 33220925. Pubmed Central PMCID: PMC7648511. Epub 2020/11/23.
7. Zubieta-Calleja G., Zubieta-DeUrioste N.. Pneumolysis and "Silent Hypoxemia" in COVID-19. *Indian J Clin Biochem* 2021;**32**:112–16 Nov 9PubMed PMID: 33191989. Pubmed Central PMCID: PMC7652053. Epub 2020/11/17. doi:10.1007/s12291-020-00935-0.
8. Erinmez M., Manay A.B., Zer Y. Investigation of an outbreak of Elizabethkingia meningoseptica on a pediatric intensive care unit. *GMS Hyg Infect Control* 2021;**16** Doc19. PubMed PMID: 34113537. Pubmed Central PMCID: PMC8167457. Epub 20210531. doi:10.3205/dgkh000390.

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Prolonged viral shedding in severely ill patients infected with SARS-CoV-2 Delta variants: A retrospective cohort study

Dear Editor,

Non-pharmaceutical interventions (NPIs) and vaccination programs have played crucial roles in mitigating a novel coronavirus disease 2019 (COVID-19) pandemic. The isolation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected individuals is one of the important and effective NPIs[1]. Many studies revealed that patients with mild-to-moderate illness confirmed with SARS-CoV-2 could not be infectious beyond 10 days after symptom onset[2]. Therefore, the guidelines were published for releasing COVID-19 patients from isolation 10 days after symptom onset,

plus at least 3 additional days without symptoms. For severely ill COVID-19 patients, extending the duration of isolation and precautions up to 20 days after symptom onset and after resolution of fever and improvement of other symptoms may be warranted.

The emergence of SARS-CoV-2 variants of concern (VOCs) harboring mutations in the spike (S) protein has raised concerns about higher transmissibility and potential immune escape after vaccination and natural infection. The B.1.617.2 (delta) variant was detected in India in December 2020. New variants and SARS-CoV-2 vaccination strategies may influence the duration of viable virus shedding in confirmed patients[3]. In addition, corticosteroid and interleukin (IL)−6 blockade, tocilizumab, which have been recommended regimens in severe patients, may be associated with prolonged viral shedding[4]. Therefore, any recommendations which use a fixed number of days should incorporate various influences on viral shedding such as host susceptibility, vaccination status, and virus strains. In addition, more studies are needed to fully understand virus transmission related to the delta variant among severe patients. Since virus culture is important to assess the viability, and could be a surrogate for transmissibility, we aimed to identify the transmissibility of SARS-CoV-2 in severe patients who were infected with the delta variant in comparison to the original virus (Wuhan-hu-1).

Twenty-six patients who were hospitalized and diagnosed with delta variant infections between June and August 2021 were consecutively enrolled as the delta variant cohort. The cohort was compared to the original strain cohort, which comprised twenty-one original virus-infected patients who were consecutively hospitalized between February and June 2020. It was a single center cohort study at a referral hospital where COVID-19 patients at higher risk of severe infection were transferred, and antiviral therapy as well as other medication, including immunomodulator, was administered based on the guideline available at the time and clinical judgement. Our team has previously reported the result of the original virus culture to suggest the duration of transmissibility of the virus[5]. According to the study, samples from patients with original virus were collected 1, 3, 5, 7, 10, and 14 days after admission. In delta variant patients, samples from nasopharyngeal (NP) swabs and sputum were obtained at times decided by an attending physician. The study was approved by the Institutional Review Board of Severance Hospital (4–2020–0076) and written informed consent was obtained from all subjects.

We defined severity of the COVID-19 infection that mild patients required hospitalization without oxygen therapy, moderate patients required low flow oxygen therapy, severe patients required high-flow oxygen therapy, and critically ill patients required mechanical ventilation or extracorporeal membrane oxygenation. The methods of confirmation, identification and culture of SARS-CoV-2 methods were described in Supplementary method.

In the delta variant cohort, the patients whose samples were collected beyond 14 days was further divided in two groups: “late clearance (LC)” group which is defined as virus culture positive beyond 14 days after symptom onset and “early clearance (EC)” whose samples beyond 14 days but were culture negative.

The demographics and clinical characteristics of COVID-19 patients who were consecutively enrolled during different period are shown in Supplementary Table. The median age of patients infected with delta variants and original strains was 58 and 72, respectively. 77% of the delta variant group had a severe form of infection requiring high flow oxygen therapy, while only 28.6% patients were severe in original strain group. In addition, according to the guideline at the time, only 28.6% of patients with the original virus received the antiviral agent, remdesivir, but all delta variant patients were administered remdesivir. For the purpose of anti-inflammation, all and 24 out of 26 (92.3%) patients infected with delta variants whereas 8 out of 21 (38.1%) and no patients with original virus received steroids and IL-6 blockade, respectively.

Table 1
Demographics of late clearance (LC) and early clearance (EC) groups in delta variant confirmed patients.

	LC (n = 8)	EC (n = 13)	P-value
Sex, male	3 (37.5)	12 (92.3)	0.014
Age, years, median [IQR]	63 [56–73.3]	58 [50–63]	<0.001
Comorbidities			
HTN	4 (50)	2 (23.1)	0.346
DM	2 (25)	2 (15.4)	0.618
Heart failure	1 (12.5)	2 (15.4)	1.0
Malignancy	1 (12.5)	1 (7.7)	1.0
Immunosuppressed	0	1 (7.7)	1.0
Severity[1]			0.67
Moderate	2 (25)	4 (30.8)	
Severe	2 (25)	4 (30.8)	
Critically ill	4 (50)	5 (38.5)	
Medication			
Corticosteroid	8 (100)	13 (100)	1.0
steroid total dose[2]	197.9	190	0.75
Tocilizumab	8 (100)	13 (100)	1.0
Mortality	3 (37.5)	0	0.042

Values were presented as numbers (%), unless other described.
IQR=interquartile range; HTN=hypertension; DM=diabetes mellitus.
[1] moderate patients required low flow oxygen therapy, severe patients required high flow oxygen therapy, and critically ill patients required mechanical ventilation or extracorporeal membrane oxygenation.
[2] The doses were adjusted to equivalent doses of prednisolone (mg).

In the original virus group, 29 and 28 samples were from NP and sputum samples and 9 (31.0%) and 13 (46.4%) were culture-positive in collected NP and sputum samples, respectively. In the delta virus group, 67 and 68 samples, and 22 (32.8%), and 20 (29.4%) samples were cultured in NP and sputum, respectively. Cyclic threshold (Ct) values of collected samples from the delta variant and original SARS-CoV-2 virus with culture tests is described in Fig. 1. We provided a Ct value for E gene of copy number quantification.

In the original virus group, all viable viruses in the NP samples was seen within 10 days after symptom onset, and three (10.7%) were culture positive beyond 10 days in the sputum samples[5]. In the delta variant group, seven (10.4%) and nine (13.2%) samples presented viable virus beyond 10 days in NP and sputum samples, respectively, implying delta-variant patients showed longer duration of viable virus in both nasopharyngeal and sputum samples than the original virus. In addition, Ct values of four from culture-positive samples in delta variant group were over 30 in culture-positive samples. One patient who was hospitalized with mechanical ventilation produced a viable virus from a NP swab 21 days after symptom onset.

In ad hoc analysis of the delta variant group, the demographics of both LC and EC group is described in Table 1. All patients received steroid and IL-6 blockade, and the doses of corticosteroid were similar in both groups. In the LC group, critically ill patients represented half of the patients, but the severity between groups did not show statistical differences. The median age in the LC group was significantly higher than that in the EC group ($p<0.001$) and female has high portion in the LC group ($p = 0.014$). Epidemiologic studies show that the delta variant might be more virulent, increasing risk of hospitalization, intensive care unit (ICU) admission, and death[6]. In our study, most of the delta variant-infected patients had received critical care in ICU, ten of whom needed ventilation support and three, extracorporeal membrane oxygenation. They produced more proportion of replication-competent virus than original virus-infected patients beyond 10 days after symptom onset (10.4% vs. 0, 13.23% vs. 10.7% in nasopharyngeal and sputum samples, respectively), implying late viral clearance; one

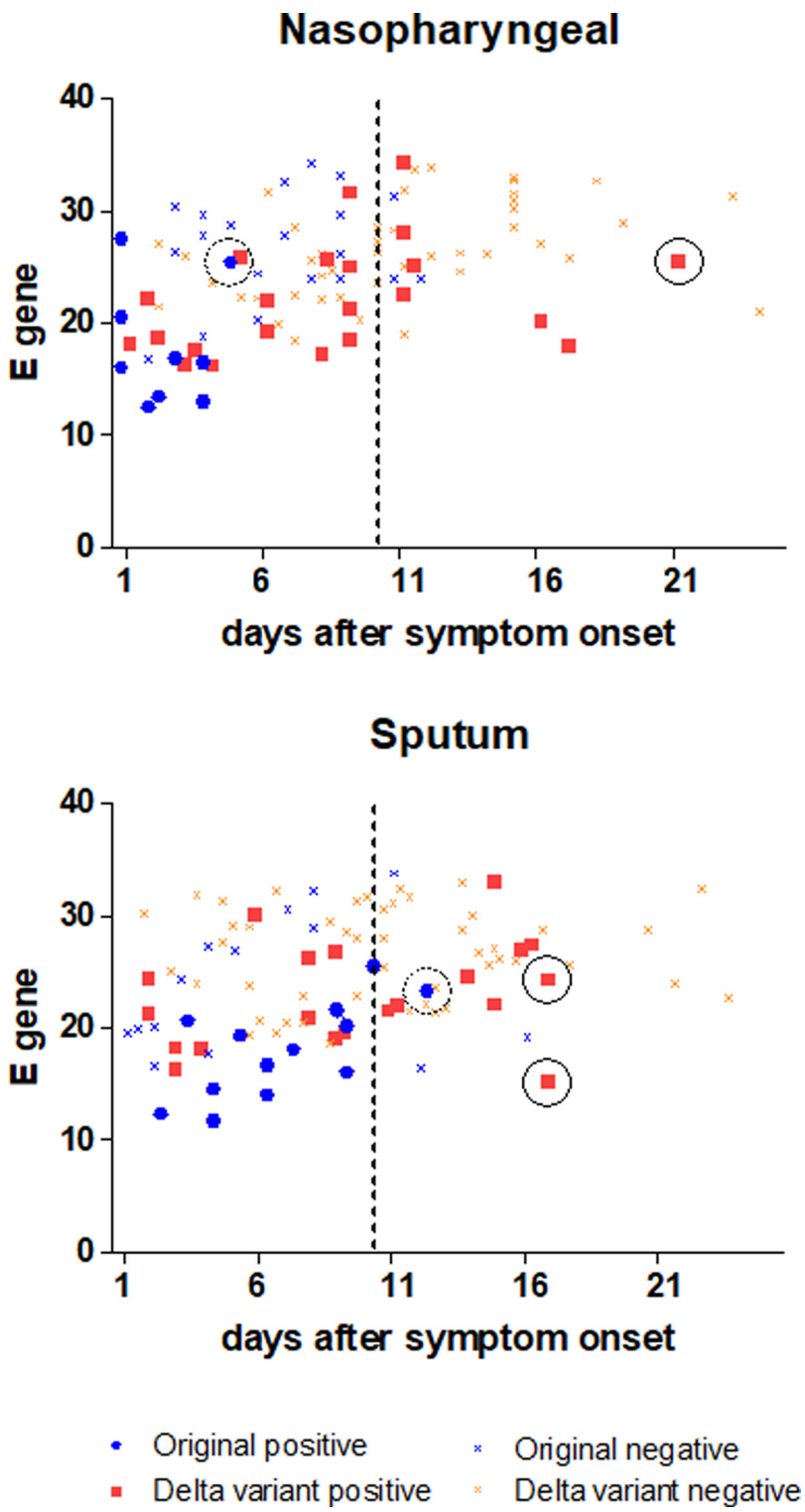

Fig. 1. Cyclic threshold (Ct) value of collected samples with culture tests at time point after symptom onset in (A) nasopharyngeal, (B) sputum samples. Negative and positive mean culture-negative and culture-positive, respectively. The dashed lines indicate the general time of releasing COVID-19 patients who are not seriously ill, the solid circles show the latest presence of the viable Delta variant, and the dotted circles represent the latest presence of the viable original virus.

critically ill patient had viable virus in the sputum 15 days after symptom onset with a Ct value of 32.96. Few samples were obtained 20 days after symptom onset according to the study protocol, but a replication-competent virus was confirmed 21 days after symptom onset from a nasopharyngeal sample of 77-year-old male with underlying bladder cancer and coronary artery disease. Because of this culture result, the patient could have infected others via the viable virus beyond 20 days.

A recent study suggested SARS-CoV-2 viral dynamics for some VOCs, indicating that peak Ct was lower, but posterior trajectories had higher proportions of Ct count less than 15 in the delta variant compared to the original or other variant of SARS-CoV-2[7]. Other study revealed that individual infections during which viral replication is initially fastest generate the highest peak viral load and see the slowest viral clearance, but vaccinations accelerate viral clearance[3]. In our study, only three patients received partial

vaccination (primer dose of vaccination), so other factors such as strains, medication could impact the viral clearance. Although interleukin (IL)−6 blockade is associated with clinical improvements for COVID-19 patients[8], it could suppress pathogenic T-cells and inflammatory monocytes, while inducing a reduction in the peripheral memory B cells and suppressing the viral clearance[4]. Therefore, compromised immunity due to immunomodulatory combination therapy for severe infection may allow the virus to continually replicate and progressively accrue genetic diversity[9]. In addition, older age and female are related to prolonged viral shedding in subsequent analysis, which is consistent with the previous results and difference in immune responses to the infection[10]. In fact, some severe patients confirmed with delta variant have prolonged viral shedding regardless of viral load of the samples, indicating that Ct values would not be specific for viability of the delta variant SARS-CoV-2.

One limitation of our study is, since it is an observational cohort study, two separate cohorts show different baseline characteristics, which are not comparable to analyze. In addition, the relatively small samples may limit the ability to verify the difference between the delta variant and other SARS-CoV-2. Stratified matching with a large sample size would be warranted to determine the isolation period and risk factors in severe patients. A culturable virus does not necessarily mean a transmissible virus. Lastly, in real-world, large-scale vaccination programs and emerging different variants (eg, Omicron variant) could complicate the viral kinetics and the timing of when to end isolation. However, our study suggests that severe form of SARS-CoV-2 infected patients, in particular, who were administered immunomodulators such as IL-6 blockade or old, should be considered for longer periods of isolation and precautions on the basis of virus culture results.

Conflict of interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.003.

Reference

1. Jeong Y.D., Ejima K., Kim K.S., Iwanami S., Bento A.I., Fujita Y., et al. Revisiting the guidelines for ending isolation for COVID-19 patients. *Elife* 2021;**10**.
2. Walsh K.A., Spillane S., Comber L., Cardwell K., Harrington P., Connell J., et al. The duration of infectiousness of individuals infected with SARS-CoV-2. *J Infect* 2020;**81**(6):847–56.
3. Singanayagam A., Hakki S., Dunning J., Madon K.J., Crone M.A., Koycheva A., et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *Lancet Infect Dis* 2021.
4. Cogliati Dezza F., Oliva A., Cancelli F., Savelloni G., Valeri S., Mauro V., et al. Determinants of prolonged viral RNA shedding in hospitalized patients with SARS-CoV-2 infection. *Diagn Microbiol Infect Dis* 2021;**100**(2):115347.
5. Baek Y.J., Lee Y.J., Yoon J.S., Sohn Y., Cho Y., Kim M.H., et al. Duration of culturable SARS-CoV-2 within different specimens among mild and severe COVID-19 patients: a longitudinal study. *J Infect* 2021;**83**(1):e29–31.
6. Fisman D., Tuite A. Progressive Increase in Virulence of Novel SARS-CoV-2 Variants in Ontario, Canada, February to June 2021. MedRxiv. 2021.
7. Kissler S.M., Fauver J.R., Mack C., Tai C.G., Breban M.I., Watkins A.E., et al. Viral Dynamics of SARS-CoV-2 Variants in Vaccinated and Unvaccinated Persons. *N Engl J Med* 2021;**385**(26):2489–91.
8. Luo L., Luo T., Du M., Mei H., Hu Y.. Efficacy and safety of tocilizumab in hospitalized COVID-19 patients: a systematic review and meta-analysis. *J Infect* 2022;**84**(3):418–67.
9. Koeckerling D., Barker J.. Accelerating the Evolution of Severe Acute Respiratory Syndrome Coronavirus 2: a Risk of Combining Dexamethasone and Tocilizumab for Severe Coronavirus Disease 2019. *J Infect Dis* 2021;**224**(6):934–7.
10. Long H., Zhao J., Zeng H.-.L., Lu Q.-.B., Fang Ll-Q, Wang Q., et al. Prolonged viral shedding of SARS-CoV-2 and related factors in symptomatic COVID-19 patients: a prospective study. *BMC Infect. Dis.* 2021;**21**(1):1–10.

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Prevalence and genetic diversity of Dabieshan tick virus in Shandong Province, China

Dear Editor,

Ticks are ectoparasites of the phylum Arthropoda that can transmit zoonotic pathogens to humans and are second only to mosquitoes as carriers of infectious diseases.[1] Tick-borne diseases have been a major public health threat in recent decades,[2] with the majority viral pathogens. Most tick-borne viral pathogens discovered to date are RNA viruses, which can evolve at a relatively high rate to adapt to environmental changes.[3] A growing number of novel tick-borne viruses have been discovered due to the employment of high-throughput sequencing technology. For example, the severe fever with thrombocytopenia syndrome virus (SFTSV) was first reported in the Henan province of China in 2010, where human infection leads to a severe hemorrhagic disease.[4] Jingmen tick virus (JMTV) in the family *Flaviviridae* was identified in Hubei Province, China.[5] More recently, Alongshan virus (ALSV) belonging to a currently unclassified Jingmenvirus group in the family *Flaviviridae* was found in Northeast China.[6] ALSV is closely related to JMTV and a subset of patients with ALSV infection present with fever and headache.[6]

Dabieshan tick virus (DBTV) is an emerging tick-borne virus in the family *Phenuiviridae*.[7] DBTV was first discovered in Wuhan in 2015, and subsequently found in Zhejiang and Shandong

Abbreviations: DBTV, Dabieshan tick virus.

provinces.[8-10] suggesting a possibly wider circulation. However, only very limited genomic information of this novel virus is available.[10] Therefore, prevalence and genetic characteristics of DBTV remain obscure and warrant further investigation.

In the present study, a total of 739 Ixodid ticks including 30 free-living individuals and 709 parasitic individuals were collected from Shandong Province, China between May and July 2019. These ticks were collected from Taian (n=273), Rizhao (n=38), Weifang (n=18), and Linyi (n=410) cities, respectively (Fig. 1). Specifically, 270 ticks collected from Taian and 18 ticks from Weifang were parasitizing goats; three ticks from Taian, 38 ticks from Rizhao and 90 ticks from Linyi were feeding on dogs; 30 ticks from Linyi were captured on grasslands and 290 ticks parasitized cattle (Fig. 1). These samples were merged into 46 pools based on morphology, collection date, location and host (Table S1).

High-throughput sequencing revealed the presence of DBTV in 30 libraries (Supplementary materials and methods). The 30 libraries belonged to all four sampling cities: Taian (n=15), Rizhao (n=3), Linyi (n=11), and Weifang (n=1). The ticks of SDTA5, SDTA12, and SDRZ2 were characterized as *Rhipicephalus turanicus* and those from SDTA6 and SDLY16 were *Haemaphysalis concinna*. The tick species from pools SDTA1, SDTA3, SDTA4, SDTA7, SDTA8, SDTA11, and SDTA13 were characterized as mixed *Haemaphysalis longicornis* and *R. turanicus*. The ticks from the remaining libraries were all characterized as *H. longicornis* (Table S1).

RT-qPCR showed that 28 out of the 30 libraries were positive for DBTV, with cycle threshold (*Ct*) values between 21.05 and 30.62. The number of read-pairs mapping to the L segment ranged from 566 to 68,784, with the average depth ranging from 8.73±8.27 to 1054.57±543.97, and 60 to 10,616 read-pairs were mapped to the S segment, with an average depth ranging from 3.77±2.61 to 890.5±505.33 (Table S2). To further confirm the degenerate bases and to differentiate the real gene variants in the sequencing pool (Table S3), specific primers were designed for Sanger sequencing (Table S4). Finally, we obtained a total of 33 L segment and 31 S segment sequences of DBTV after integrating results from Sanger sequencing and high-throughput sequencing. The L and S genes of DBTV described here ranged from 6485–6544 nt and 1732–1786 nt in length, respectively. The open reading frame (6447 nt) of the L gene encoded an RNA-dependent RNA polymerase (RdRp) of 2149 amino acids in length, whereas the open reading frame (825 nt) of the S gene encoded a nucleocapsid protein of 275 amino acids in length.

Sequence comparison revealed that the genome sequences of DBTV described here were highly similar to the three previously reported DBTV strains in China. The L gene sequence of SDLY16 shared the highest sequence identity with MT413430|DTV|SD|China|2017 (99.33%). Among the 33 viral strains in our study, SDRZ1 exhibited the lowest nucleotide identity with MN723843|DTV|ZS-DBS-2018|China|2016_06_12 (97.23%). All the novel L gene sequences in our study shared >97% sequence identity (97.94%–99.98%) with each other. In addition, the RdRp proteins exhibited 99.07%–100% identity with each other. With respect to the S gene, SDTA15b shared the highest sequence identity with MT413431|DTV|SD|China|2017 (99.80%), and SDTA13b exhibited the lowest nucleotide identity with KM817733|DTV|D3|China|2013 (97.48%). Viral S gene sequences shared 97.39%–99.86% nucleotide identity with each other and 99.27%–100% amino acid similarity with each other.

Phylogenetic analysis showed that the 33 L segments of DBTV could be classified into five well-supported clus-

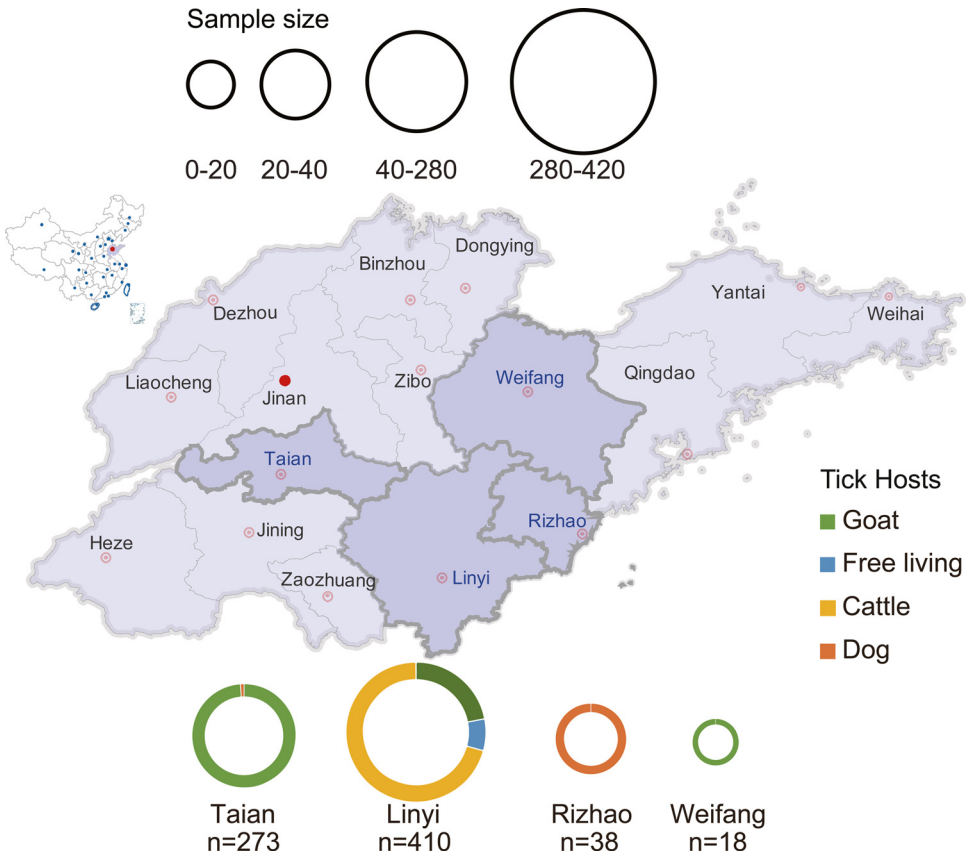

Fig. 1. Distribution of the tick samples collected in Shandong Province, China.
The four sampling cities (Taian, Weifang, Linyi, and Rizhao) in Shandong Province are highlighted on the map. The number of ticks collected from each city is presented. The host types of ticks were marked by different colors: green for goat, yellow for cattle, orange for dog, and blue for free living.

ters (Fig. 2a). 16 virus strains from Taian formed cluster L1. MT413430|DTV|SD|China|2017 formed a relatively more divergent cluster L2. However, two strains from Taian (SDTA8 and SDTA15–1), two strains from Rizhao (SDRZ2–2 and SDRZ3–1), one strain from Weifang (SDWF1) and two strains found in Wuhan (KM817666|DTV|D3|China|2013) and Zhoushan (MN723843|DTV|ZS-DBS-2018|China|2016_06_12) formed cluster L5. Notably, eight strains from Linyi (SDLY1, SDLY7, SDLY8, SDLY14, SDLY16, SDLY17, SDLY22, and SDLY23) grouped together with two strains from Rizhao (SDRZ2–1 and SDRZ3–2), forming cluster L4. In addition, SDRZ1 and SDLY3 formed cluster L3 (Fig. 2a). In the S gene tree, the 31 novel sequences of DBTV formed two clusters. The 15 virus sequences from Taian formed an independent cluster S2 (Fig. 2b). All the other novel sequences fell within cluster S1, with the three previously reported virus strains.

In summary, we have described 33 L and 31 S gene sequences of DBTV from various ticks collected in different regions of Shandong Province, China, which greatly enriches the genomic information of DBTV. Our results indicate that DBTV is prevalent in Shandong Province, and it has evolved into different phylogenetically distinct lineages. Although there have not been human or animal cases of DBTV reported to date, given its wide distribution, pathogenesis studies are urgently needed to determine the risk of mammalian and human infection.

Data and code availability

The raw data of these 28 libraries have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA797715 (SRA accession numbers: SRR17794527-SRR17794554). The genome sequences of 33 L and 31 S segments of DBTV generated in this study have also been deposited in the GenBank and assigned accession numbers OM368029-OM368061 and OM367998-OM368028, respectively.

Author contributions

Conceptualization, Funding acquisition and Supervision: W.S., and Z.Z.; Resources: H.Z., R.Z., G.Y., M.C., and Z.Z.; Formal analysis and Methodology: C.M., H.Z., M.C., and L.Y.; Data curation: C.M., H.Z., J.L., and W.S.; Writing- original draft, review & editing: C.M., H.Z., Z.Z., M.J.C., and W.S.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.002.

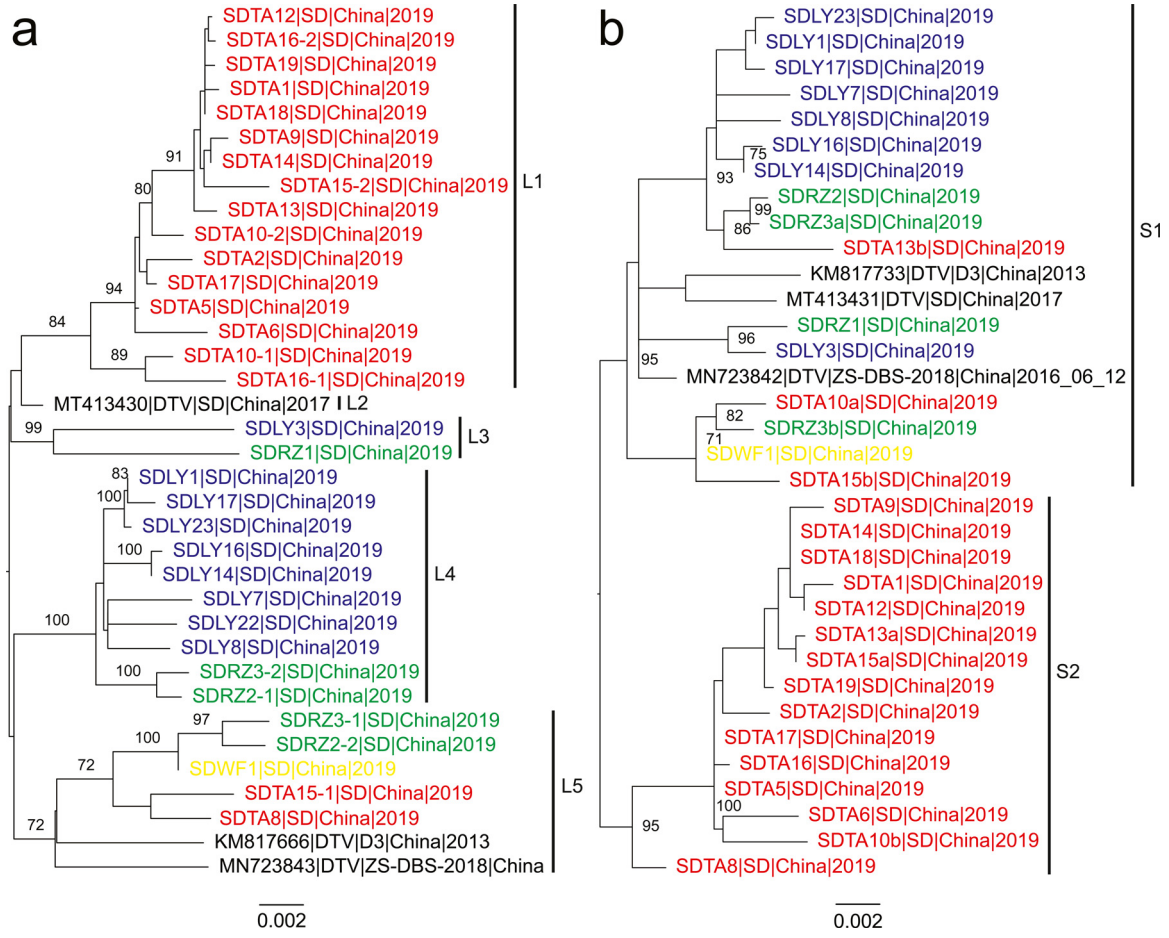

Fig. 2. Phylogenetic analysis of all known Dabieshan tick viruses.
Phylogenetic trees of the L (a) and S (b) gene sequences of DBTV were generated by using RAxML with 1000 bootstrap replicates. The viral strains found in our study are highlighted with different colors according to the sampling location: red for Taian, blue for Linyi, green for Rizhao, and yellow for Weifang.

References

1. Dantas-Torres F., et al. Ticks and tick-borne diseases: A One Health perspective. *Trends Parasitol* 2012;**28**(10):437–46.
2. Kilpatrick A.M., et al. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* 2012;**380**(9857):1946–55.
3. Papa A., et al. Novel phlebovirus detected in Haemaphysalis parva ticks in a Greek island. *Ticks Tick Borne Dis* 2017;**8**(1):157–60.
4. Liu Q., et al. Severe fever with thrombocytopenia syndrome, an emerging tick--borne zoonosis. *Lancet Infect Dis* 2014;**14**(8):763–72.
5. Qin X.C., et al. A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors. *Proc Natl Acad Sci U S A* 2014;**111**(18):6744–9.
6. Wang Z.D., et al. A new segmented virus associated with human febrile illness in China. *N Engl J Med* 2019;**380**(22):2116–25.
7. Shi J., et al. Tick-Borne Viruses. *Virol Sin* 2018;**33**(1):21–43.
8. Li C.X., et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* 2015;**4**:e05378.
9. Shao L., et al. Identification of recently identified tick-borne viruses (Dabieshan tick virus and SFTSV) by metagenomic analysis in ticks from Shandong Province, China. *J Infect* 2020;**81**(6):973–8.
10. Zhu C., et al. Distribution and phylogenetic analysis of Dabieshan tick virus in ticks collected from Zhoushan, China. *J Vet Med Sci* 2020;**82**(8):1226–30.

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SARS-CoV-2 rapid test versus RT-qPCR on noninvasive respiratory self-samples during a city mass testing campaign.

Dear Editor,

A few months ago, Baro et al. published in this Journal an interesting study comparing different rapid lateral-flow tests (RLFT) dedicated to the direct diagnosis of SARS-CoV-2 in nasopharyngeal aspirates in a large population of asymptomatic subjects in Catalonia, Spain. They showed that the analytical performances of some of them were compatible with their use in routine, notably with an objective of mass screening.[1] In accordance with these observations, we conducted in February 2021 a city-scale screening to evaluate the prevalence of SARS-CoV-2 infection in the town of Saint-Etienne (the 14th most populated city in France in 2018 with 173,000 inhabitants) where the circulation of the virus had exhibited the highest incidence of positive cases in France in October-November 2020. By contrast to other mass testing campaigns,[1-7] the choice was done of noninvasive tests based on salivary specimens. This vast operation led to the inclusion of more than 7000 individuals within a single week, which represents approximately 4% of the whole indoor population of Saint-Etienne.

We report the performance of the COVID19 Speed-Antigen Test (Biospeedia, France) on self-sampled material combining anterior nose and saliva, by comparison to RT-qPCR on saliva, in the context of this campaign. Data regarding acceptability of the different sampling methods were recorded by optional questionnaire. Adults and children over 10 years of age able to provide a self-sample were eligible for inclusion, after oral approval of an informative note by the subject and/or his/her guardian. Participants provided information on demographic characteristics, occupation and symptoms (protocol IDRCB number 2021-A00390–41). They were invited optionally to complete an on-line questionnaire (Limesurvey® software, Hamburg, Germany) including scales in relation with the acceptability of the different modes of specimen collection.

Participants were asked to collect at least 3 ml of saliva in a 50 ml sterile plastic tube more than 30 min after drinking, eating, smoking, brushing teeth or rinsing teeth. This sample was used for RT-qPCR analysis that served as gold standard in this study. In parallel, 2 drops of saliva were transferred into a second tube previously filled with 8 drops of the buffer of the RFLT kit. An anterior nasal sample was then performed by the participant. The content of this swab was then discharged in the tube containing saliva and buffer. This mixture was used to perform the COVID19 Speed-Antigen Test (Biospeedia, France) by the assistants specially

Abbreviations: RLFT, rapid lateral-flow test; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; C_T, cycle threshold; CI_{95}, confidence interval 95%; VOC, variant of concern.

Table 1
Variables associated to the positivity of the rapid lateral flow test (RLFT).

	PCR+/RLFT+ (N=98) N (%)	PCR+/RLFT-(N=67) N (%)	Crude Odds Ratio ($CI_{95\%}$)	Adjusted Odds Ratio ($CI_{95\%}$)	Adjusted *P* value
C_T value					< 0.001
- >28	8 (15.4)	44 (84.6)	-	-	
- ≤28	90 (79.6)	23 (20.4)	21.52 (9.34–55.30)	23.96 (7.75–94.03)	
Typing					0.870
- non variant	49 (57.6)	36 (42.4)	-	-	
- variant	48 (70.6)	20 (29.4)	1.76 (0.90–3.51)	1.08 (0.41–2.83)	
Covid-19 symptoms					0.991
- no	36 (48.6)	38 (51.4)	-	-	
- yes	32 (66.7)	16 (33.3)	2.11 (1.00–4.56)	1.01 (0.38–2.65)	

C_T: cycle threshold. CI_{95}: confidence interval 95%.

trained for this technique on the different sites of the mass testing campaign, according to the manufacturer's instructions.

The RT-qPCR tests were performed in three PCR independent platforms located at the University Hospitals of Lyon (4278 specimens) and Clermont-Ferrand (1291 specimens) and at the bioMérieux Research Laboratory of Grenoble (1068 specimens). Invalid tests were submitted to a second round of extraction/amplification; in case of two successive failures, the result was declared invalid. As different RT-qPCR tests were used, a correction was done to homogenate the cycle threshold (C_T) values according to the concordance table of the French Society of Microbiology.[8] Positive samples were typed by the Lyon laboratory for the identification of variant strains of SARS-CoV-2.

From February 22 to 28 of 2021, 7020 subjects participated to the mass testing campaign organized in Saint-Etienne but 381 individuals were not included, mainly for young age ($N = 132$) or missing specimens or data ($N = 249$). From the 6639 included volunteers, 56% were female and the median age was 48 years (interquartile range: 30–64); 90.5% of participants were asymptomatic with regard to SARS-CoV-2 infection.

Overall, 165 participants were tested positive by RT-qPCR and 114 by RLFT. The number of positive specimens by RLFT was almost significantly lower in asymptomatic than in symptomatic individuals ($P = 0.05$). The sensitivity, specificity, positive predictive value and negative predictive value of RLFT were 59.4 (CI_{95}: 51.5–67.0), 99.8 (CI_{95}: 99.6–99.9), 86.0 (CI_{95}: 78.2–91.8) and 99.0 (CI_{95}: 98.7–99.2), respectively, by reference to RT-qPCR. The sensitivity of RLFT dropped to 48.6% (CI_{95}: 36.9–60.6) when only asymptomatic participants were considered. This latter sensitivity was close to that observed with the best kits evaluated by Baro et al.[1] and a bit superior to that observed with similar commercial tests in Liverpool (40.0%),[2] in the Pima county of Arizona (35.8%)[3] or in Kuwait City (42.3% with the first RFLT and 30.6% with the second one).[4]

The 165 strains recorded by RT-qPCR were submitted to molecular typing: 13 were not typeable, 85 belonged to the original phenotype whereas 68 exhibited a variant of concern (VOC) profile (66 Alpha and 2 Beta or Gamma). The median C_T value was significantly higher in variant than in non-variant isolates (21.8 *vs.* 25.7, $P < 0.001$ by Wilcoxon-Mann-Whitney test).

A strong inverse correlation (Kendall's τ coefficient of -0.910; $P < 10^{-8}$) was observed between the C_T value measured by RT-qPCR and RLFT sensitivity. The sensitivity dropped from 100% to 15.4% for C_T values ≤ 18 and >28, respectively (**Fig. 1**). Three variables were associated to RLFT positivity: low C_T value, detection of SARS-CoV-2 variant and presence of Covid-19 symptoms; however, after multivariate analysis, the C_T value remained the sole explicative variable (**Table. 1**).

Different criteria of acceptability (pain, sampling convenience) of the tests were assessed on about two-thirds of the tested population who accepted to fill out the corresponding questionnaire. In agreement with others,[9,10] our results (data not shown) indicate that salivary auto-tests (associated or not to anterior nose) were considered less painful, especially in women and young people, and as convenient as conventional nasopharyngeal swabbing.

In agreement with others using similar RLFT,[1-7] this study comforts the pertinence of the COVID19 Speed-Antigen Test to be used for detecting through mass testing asymptomatic individuals at high risk to disseminate COVID-19 due to their high viral load. The result availability within minutes could allow the rapid implementation of isolation measures and immediate set-up of contact tracing. The use of samples combining anterior nose and saliva is another innovation tested in the study. We confirm the feasibility and the excellent acceptability of noninvasive self-sampling by asymptomatic volunteers involved in large campaigns of screening.

AutoCov study group composition (city)

Amandine Baudot (Saint-Etienne), Geneviève Billaud (Lyon), Thomas Celarier (Saint-Etienne), Laure Choupeaux (Paris), Hélène Chabrolles (Clermont-Ferrand), Laura Cinieri (Saint-Etienne), Constance Delaugerre (Paris), Christine Forissier (Saint-Etienne), Emilie Frobert (Lyon), Alexandre Gaymard (Lyon), Cécile Henquell (Clermont-Ferrand), Isabelle Martin (Saint-Etienne), Agathe Mattei (Saint-Etienne), Audrey Mirand (Clermont-Ferrand), Aline Tchaplyguine (Saint-Etienne) and Jean-Marc Treluyer (Paris).

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Authors' contributions

Conceptualization: JG, PM, TB, FC, PhB, EBN & BP. Data curation: JG, PaB, EV, SG & BP. Formal analysis: JG, PaB, EV, SG & BP. Funding acquisition: JG, PM, FC & BP. Investigation: JG, BB, RL & BP. Methodology: RL, SG, SP, AB, FM, TB, AutoCoV study group & BP Project administration: JG, FC, PhB, EBN & BP Resources: JG, FC & BPSoftware: PaB. Supervision: JG, BB, RL & BP. Validation: JG, PaB, EV, RL, SG, AB, FM, AutoCoV study group & BP. Visualization: PaB. Writing - original draft: JG, EBN & BP. Writing - revew and editing: all authors.

Conflict of Interest

None declared.

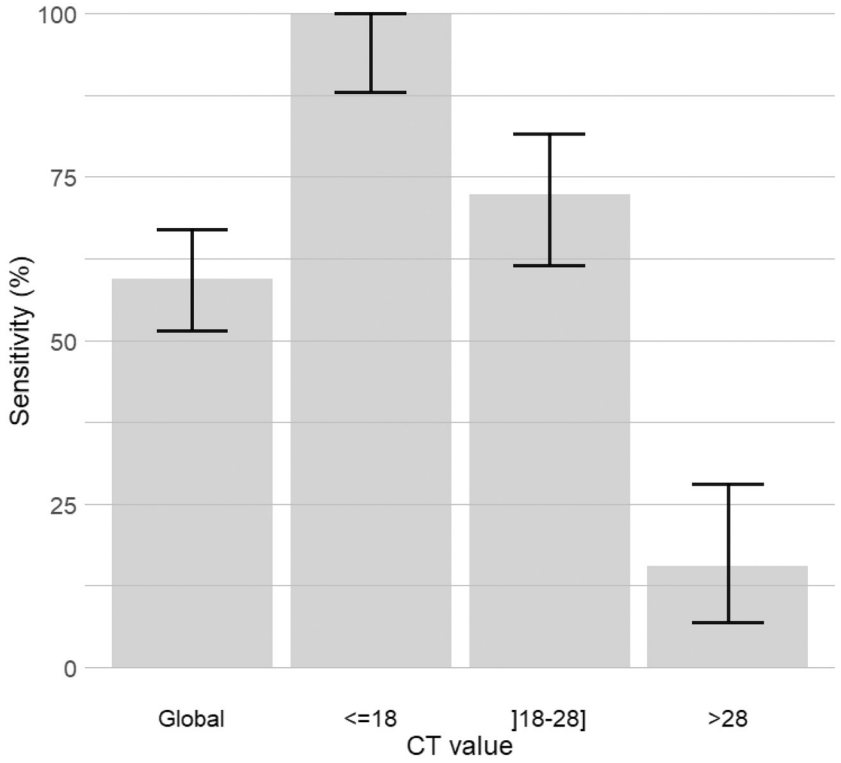

Fig. 1. Sensitivity of the rapid lateral-flow test with reference to RT-qPCR taken as gold standard. The bar on the left correspond to the overall sensitivity. Bars on the right correspond to stratified sensitivity according to the Cycle threshold (C_T) value of the RT-qPCR assay.

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References

1. Baro B., Rodo P., Ouchi D., Bordoy A.E., Saya Amaro E.N., Salsench S.V., et al. Performance characteristics of five antigen-detecting rapid diagnostic test (Ag-RDT) for SARS-CoV-2 asymptomatic infection–A head-to-head benchmark comparison. *J Infect* 2021;**82**:269–75.
2. García-Fiñana M., Hughes D.M., Cheyne C.P., Burnside G., Stockbridge M., Fowler T.A., et al. Performance of the Innova SARS-CoV-2 antigen rapid lateral flow test in the Liverpool asymptomatic testing pilot–Population based cohort study. *BMJ* 2021;**374**:n1637.
3. Prince-Guerra J.L., Almendares O., Nolen L.D., Gunn J.K.L., Dale A.P., Buono S.A., et al. Evaluation of Abbott BinaxNOW rapid antigen test for SARS-CoV-2 infection at two community-based testing sites - Pima County, Arizona, November 3-17, 2020. *Morb Mortal Wkly Rep* 2021;**70**:100–5 2021.
4. Alghounaim M., Bastaki H., Bin Essa F., Motlagh H., Al-Sabah S. The performance of two rapid antigen tests during population-level screening for SARS-CoV-2 infection. *Front Med (Lausanne)* 2021;**8**:797109.
5. Hledík M., Polechová J., Beiglböck M., Herdina A.N., Strassl R., Posch M.. Analysis of the specificity of a COVID-19 antigen test in the Slovak mass testing program. *PLoS One* 2021;**16**:e0255267.
6. Pollock N.R., Jacobs J.R., Tran K., Cranston A.E., Smith S., O'Kane C.Y., et al. Performance and implementation evaluation of the Abbott BinaxNOW Rapid Antigen Test in a high-throughput drive-through community testing site in Massachusetts. *J Clin Microbiol* 2021;**59** e00083-21.
7. Marks M., Millat-Martinez P., Ouchi D., Roberts C.H., Alemany A., Corbacho–Monné M., et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain–A cohort study. *Lancet Infect Dis* 2021;**21**:629–36.
8. Société Française de Microbiologie. Avis du 25 septembre 2020 de la Société Française de Microbiologie (SFM) relatif à l'interprétation de la valeur de Ct (estimation de la charge virale) obtenue en cas de RT-PCR SARS-CoV-2 positive sur les prélèvements cliniques réalisés à des fins diagnostiques ou de dépistage Version 4 _ 14/01/2021 Mise à jour. [cited 2022 Feb 22]. https://www.sfm-microbiologie.org/wp-content/uploads/2021/01/Avis-SFM-valeur-Ct-excre%CC%81tion-virale-_-Version-def-14012021_V4.pdf.
9. Marchant E., Ready D., Wimbury G., Smithson R., Charlett A., Oliver I.. Determining the acceptability of testing contacts of confirmed COVID-19 cases to improve secondary case ascertainment. *J Public Health* 2021;**43**:e446–52.
10. Hirst J.A., Logan M., Fanshawe T.R., Mwandigha L., Wanat M., Vicary C., et al. Feasibility and acceptability of community Coronavirus Disease 2019 testing strategies (FACTS) in a University setting. *Open Forum Infect Dis* 2021;**8**:ofab495.

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Decrease of carbapenemase-producing Enterobacteriaceae incidence during the first year of the COVID-19 pandemic

Dear Editors,

We read with interest the article by Lemenand et al. who showed a decrease in the proportion of extended-spectrum beta-lactamase among *E. coli* infections in primary care and nursing home during the first year of the coronavirus disease 2019 (COVID-19) pandemic in France.[1] Several factors may have contributed to the decrease of antimicrobial resistance: the reinforcement of hygiene measures in the general population (hand hygiene, limitation of gatherings during lockdown periods), the decrease of antibiotic consumption in primary care, the limitation of the international travel and the increased awareness of healthcare workers' (HCWs) to comply with infection control measures and hand hygiene.

On the other hand, in hospitals, some factors could have contributed to worsen the spread of antibiotic resistance during the COVID-19 pandemic: increase of bacterial infections, a higher antibiotic use in hospital, a more important proportion of patients requiring hospitalization in intensive care, overcrowding in hospitals and HCWs fatigue after months of COVID-19 surge.[2–6]

The aim of our observational study was to evaluate the impact of the COVID-19 pandemic, which began in January 2020 in France,[7] on the evolution of carbapenemase-producing Enterobacteriaceae (CPE) incidence in the Assistance Publique–Hôpitaux de Paris (AP-HP). AP-HP, the largest public healthcare institution in France with 20,000 beds, is a network of 38 university-affiliated public hospitals, spread over Paris and its suburbs. Infection prevention and control teams (IPCT), coordinated by a multidisciplinary central IPCT,[8] are in charge of prevention and surveillance of healthcare-associated infections in each hospital.

Since 2004, AP-HP has implemented a long-term programme for CPE surveillance and control which is based on a bundle of measures to prevent cross transmission,[8] including pre-emptive isolation (contact precautions) and screening for CPE of every patient with a history of stay or hospitalization in a foreign country within the past year.

A case was defined as any patient infected or colonized with CPE and an episode as one index case, followed or not by secondary case(s). For each identification of CPE case, histories of recent hospitalization or stay abroad, the occurrence and number of secondary cases(s) were collected by the IPCT.

Data consisted of the quarterly number of CPE index cases from 2014 to 2020 in AP-HP hospitals. The time-series was decomposed

into three components: trend, seasonal and random, from 2014 to 2019 (period 1), using a linear additive model. We calculated the expected number of index cases during the quarters in 2020 with 95% prediction intervals (PI) and compared the number of quarterly observed and forecasted CPE index cases. The statistical programming language 'R' was used.

From 2014 to 2019, 2131 CPE index cases were identified, 380 in 2020, with a history of previous stay abroad in respectively 1438 (67%) and 156 (41%) cases.

Fig. 1 shows the observed and fitted time-series of the quarterly CPE index cases per 100,000 hospitalization-days (HD) in period 1 and the predicted values with 95%PI for 2020. We observed a significant linear upward trend ($p < 10^{-6}$) of +0.34 CPE cases/100,000 HD each quarter and a seasonal component with higher number in the third quarter, corresponding to summer periods ($p < 10^{-3}$). By comparing the number of observed and forecasted CPE incidence in 2020, it emerged that the observed cases did not fit the projection. Indeed, the observed CPE incidence was lower than the predicted lower limit of the 95% PI forecast from the second quarter.

Analysis of the CPE incident cases with a recent stay abroad showed a significant upward trend ($p < 10^{-6}$) of +0.17 CPE cases/100,000 HD each quarter and significant seasonality ($p < 10^{-5}$). By comparing the number of quarterly observed and forecasted CPE cases with a history of stay abroad, the observed CPE incidence was lower than the predicted lower limit of the 95% PI forecast from the second quarter of 2020 (Fig. 2A). Analysis of the CPE cases without a history of stay abroad showed a similar upward trend ($p < 10^{-6}$) of +0.17 CPE cases/100,000 HD each quarter but no seasonal component (Fig. 2B). Moreover, the number of observed CPE cases without a history of stay abroad remained in the 95% PI forecast during 2020.

The mean number of secondary cases per episode remained stable with 399 for 2131 episodes in period 1 (0.2 cases/episode) and 84 for 380 episodes in 2020 (0.2 cases/episode) which does not argue for an increase of CPE intra-hospital transmission during the pandemic.

Finally, this study shows that the annual number of index CPE cases dramatically decreased in 2020 in the largest public health institution in France. This concerned the CPE index cases with a history of stay abroad while those without continued to increase following the same trend as in previous years. This is probably the result of governmental restrictions during the pandemic which have limited international travel,[9] the most important source of CPE in countries as France with a low incidence.[10] In contrast, the evolution of the CPE incidence without a recent stay abroad does not seem to have been affected during the pandemic: the reinforcement of hygiene measures in the general population and in hospitals did not bend the incidence curve, but the modified conditions of care in hospitals did not increase the cross transmission either.

This study has several limitations. Firstly, the completeness of the data is questionable because the IPCT had little time to do these reports in 2020, even if a catch-up was made retrospectively in 2021. In addition, a decrease in CPE screening in 2020 is possible due to the overload of work in the intensive care unit and a shortage of swabs that lasted a few weeks during the first COVID-19 wave. However, the fact that the decrease in CPE cases concerned only cases with a history of stay abroad tempers these limitations.

In conclusion, our study shows a decrease in the incidence of CPE cases in our institution during the COVID-19 pandemic. It suggests that this decrease is linked to a decrease in international exchanges. This underlines that, to be effective, the fight against antimicrobial resistance will have to be considered at an international level.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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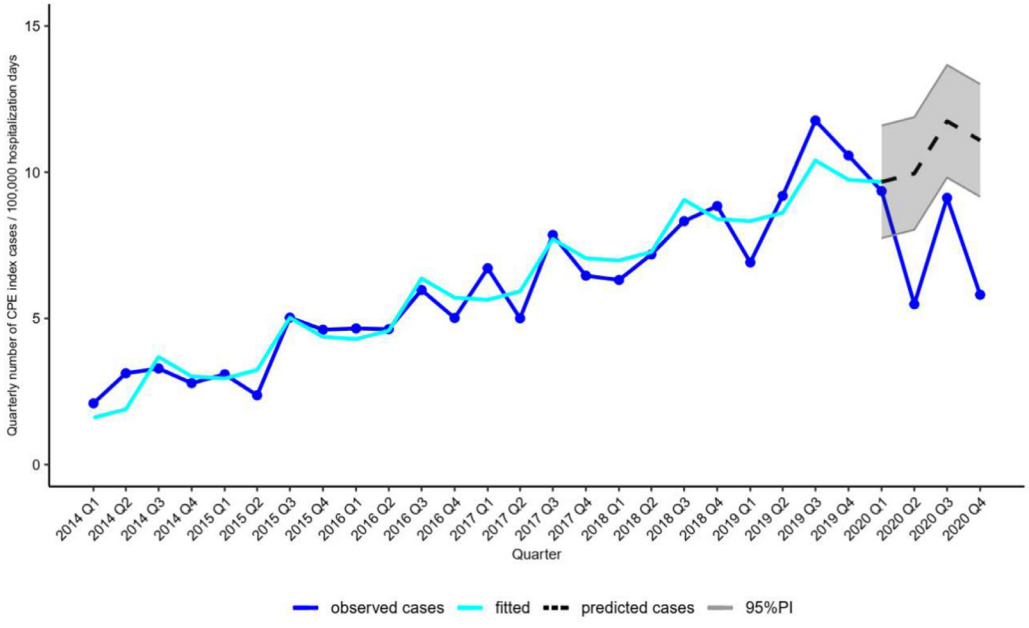

Fig. 1. Observed carbapenemase-producing Enterobacteriaceae index cases from 2014 to 2020 and 2020 forecasted cases with 95% prediction intervals.

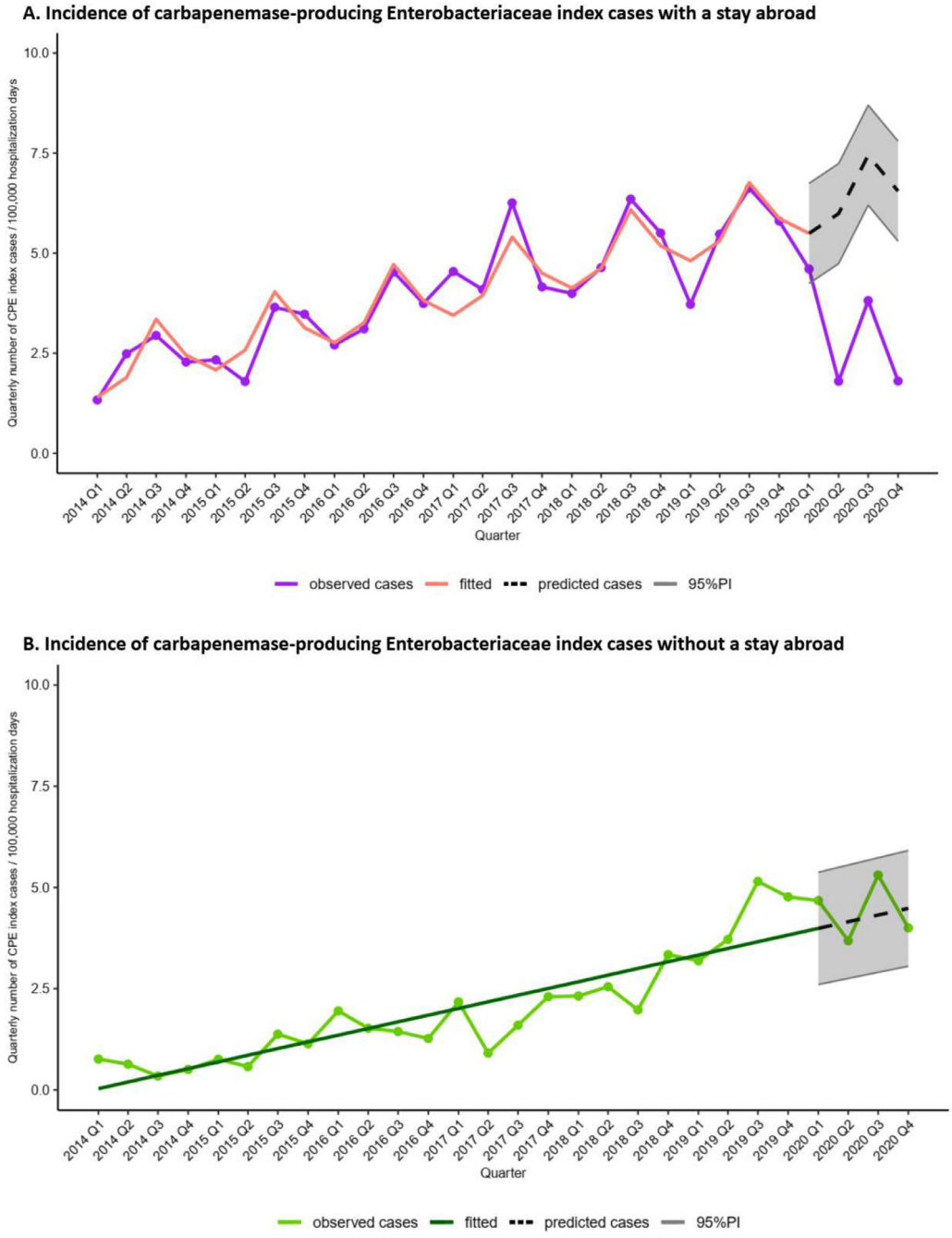

Fig. 2. Observed carbapenemase-producing Enterobacteriaceae index cases with (2A) and without (2B) a known history of stay abroad from 2014 to 2020 and 2020 forecasted cases with 95% prediction intervals.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jinf.2022.03.024.

References

1. Lemenand O., Coeffic T., Thibaut S., Colomb Cotinat M., Caillon J., Birgand G., et al. Decreasing proportion of extended-spectrum beta-lactamase among E. coli infections during the COVID-19 pandemic in France. *J Infect* 2021;**83**(6):664–70.
2. Zhou F., Yu T., Du R., Fan G., Liu Y., Liu Z., et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;**395**(10229):1054–62 Mar 28.
3. Baker M.A., Sands K.E., Huang S.S., Kleinman K., Septimus E.J., Varma N., et al. The impact of coronavirus disease 2019 (COVID-19) on healthcare-associated infections. *Clin Infect Dis* 2021:ciab688.
4. Rossato L., Negrão F.J., Simionatto S.. Could the COVID-19 pandemic aggravate antimicrobial resistance? *Am J Infect Control* 2020;**48**(9):1129–30 Sep 1.
5. Murray C.J., Ikuta K.S., Sharara F., Swetschinski L., Robles Aguilar G., Gray A., et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;**399**(10325):629–55 Feb 12.
6. Amarsy R., Trystram D., Cambau E., Monteil C., Fournier S., Oliary J., et al. Surging bloodstream infections and antimicrobial resistance during the first wave of COVID-19: a study in a large multihospital institution in the Paris region. *Int J Infect Dis* 2022;**114**:90–6 IJID Off Publ Int Soc Infect DisJan.
7. Lescure F.-X., Bouadma L., Nguyen D., Parisey M., Wicky P.-H., Behillil S., et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Infect Dis* 2020;**20**(6):697–706.
8. Fournier S., Desenfant L., Monteil C., Nion-Huang M., Richard C., Jarlier V., et al. Efficiency of different control measures for preventing carbapenemase-producing enterobacteria and glycopeptide-resistant Enterococcus faecium outbreaks: a 6-year prospective study in a French multihospital institution, January 2010 to December 2015. *Eurosurveillance* 2018;**23**(8) [Internet]Feb 22 [cited 2021 Sep 2]Available from:. doi:10.2807/1560-7917.ES.2018.23.8.17-00078.
9. Linka K., Peirlinck M., Sahli Costabal F., Kuhl E.. Outbreak dynamics of COVID-19 in Europe and the effect of travel restrictions. *Comput Methods Biomech Biomed Eng* 2020;**23**(11):710–17 Aug.
10. Voor in 't holt A.F., Mourik K., Beishuizen B., van der Schoor A.S., Verbon A., Vos M.C., et al. Acquisition of multidrug-resistant Enterobacterales during international travel: a systematic review of clinical and microbiological characteristics and meta-analyses of risk factors. *Antimicrob Resist Infect Control* 2020;**9**(1):71 May 20.

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