Asian Journal of Research in Infectious Diseases

Volume 14, Issue 3, Page 67-74, 2023; Article no.AJRID.106567
ISSN: 2582-3221

Asymptomatic Carriage of SARS-CoV-2 in a University Population in Rosario, Argentina

Juan Pablo Scapini [a,b*], María Victoria del Rosal [a], Juan Ignacio Isaac [a], Ayelen Rodriguez [b], Damian Ingaramo [a,b] and Micaela Campora [a,b]

[a] Chair of Microbiology, Instituto Universitario Italiano de Rosario, Corrientes 2009, Rosario, Argentina.
[b] SILAB, Urquiza 2725, Rosario, Argentina.

Authors' contributions

This work was carried out in collaboration among all authors. Authors JPS, MVDR and JII designed of the study. Authors MVDR and JII did the data and sample collection. Authors DI, AR and MC did the lab work. Author JPS did the interpretation of results and wrote the protocol of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2023/v14i3301

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/106567

Original Research Article

Received: 25/07/2023
Accepted: 02/10/2023
Published: 30/10/2023

ABSTRACT

Aims: To study the rates of SARS-CoV-2 asymptomatic carriage in a young, healthy and full vaccinated population of students in the medical area. Contribute to the knowledge of the dynamics of the endemic and its control.
Study Design: Cross-sectional descriptive study.
Place and Duration of Study: Faculty of Medical Sciences, Instituto Universitario Italiano de Rosario, Argentina, between August and September 2022.
Methodology: 300 students were recruited. An oropharyngeal sample was taken from all the participants who completed an online electronic survey. The sampling period corresponded to

**Corresponding author: E-mail: jpscapini@silab.com.ar;*

weeks of low viral circulation in the province. The presence of SARS-CoV-2 was investigated by real-time RT-PCR.
Results: 72.7% of the participants reported a previous infection. All the participants received at least one dose of vaccine and 56.7% completed a 3-dose schedule. Two of the 300 samples were positive for SARS-CoV-2 RNA. One was classified as presymptomatic since the individual developed compatible symptoms three days after taking the sample. The other case was the only one classified as an asymptomatic carrier, resulting in a SARS-CoV-2 portability rate of 0.33%.
Conclusion: The rate obtained for asymptomatic portability in our study was surprisingly low considering that these groups shared an average of 6 hours a day in closed environments. The risk of person to person spread in this population is extremely low and does not justify the use of masks or social distancing.

Keywords: COVID 19; SARS-CoV-2; Argentina; asymptomatic carriage.

1. INTRODUCTION

The coronaviruses are common respiratory pathogens that cause disease in humans worldwide [1]. By the end of 2019, six different coronaviruses affecting humans had been reported [2]. SARS-CoV-2 was afterwards identified as the seventh virus of the Coronaviridae family to cause infection in humans [3]. Coronaviruses that have rarely been tested systematically around the world may persist in the pharynx of asymptomatic people, representing a potential source of population immunity. Furthermore, it should be noted that systematic studies of other coronaviruses have found that the percentage of asymptomatic carriers is equal to or even higher than the percentage of symptomatic patients [4]. However, this aspect has not yet been evaluated for SARS-CoV-2. The average incubation period is 5 to 6 days, but can be up to 14 days [5]. The clinical outcome of coronavirus acute respiratory syndrome (COVID) can vary from asymptomatic to severe. Common symptoms of COVID include headache, fever, cough, fatigue, dyspnea, diarrhea, and even conjunctivitis, sometimes leading to severe SARS-like viral pneumonia (severe acute respiratory syndrome), multi-organ dysfunction, and even death [6]. In asymptomatic cases of COVID, individuals who test positive for SARS-CoV-2 nucleic acid by real-time reverse transcriptase polymerase chain reaction (RT-PCR) do not develop symptoms [5]. This population has two subpopulations: presymptomatic and true asymptomatic. Presymptomatic individuals are those without symptoms who test positive for SARS-CoV-2 and then develop symptoms, whereas true asymptomatic cases are persons who test positive but never show signs or symptoms [7]. Appropriate observations made repeatedly over a prolonged period can help differentiate between asymptomatic and presymptomatic cases [8].

Since the emergence of COVID-19, there has been much speculation about the silent transmission of the disease. Cross-sectional studies testing exposed individuals who do not exhibit symptoms often conflate asymptomatic infections with those in the presymptomatic phase, leading to substantial overestimation of asymptomatic infection. Longitudinal studies without sufficient follow-up similarly lead to overestimation of the number of asymptomatic infections. Additionally, inconsistent use of terminology has led to confusion, particularly when distinguishing infections which are silent at the time of testing from those which are truly asymptomatic. One meta-analysis, for example, incorrectly includes infections in the presymptomatic phase in the calculations of the asymptomatic percentage [9]. By contrast, several studies conducted early in the pandemic reported few asymptomatic infections, primarily due to restrictive testing criteria which focused on testing of severe cases that required hospitalization. Inaccuracy in either direction is detrimental for public health [10].

Because asymptomatic cases of COVID escape detection by public health surveillance systems, they are a challenge to potential preventive infection control measures such as self-quarantine. Moreover, the main route of SARS-CoV-2 transmission through aerosols exhaled by asymptomatic COVID carriers while breathing and talking is well documented, and cases of familial transmission through asymptomatic cases have been reported in different countries [11,12]. A recent meta-analysis reported a prevalence of asymptomatic cases of 39% (95% CI, 20.4-61.4%), without indicating vaccination status [13]. These results could not be

extrapolated to any date and location due to the kinetics of change in the susceptibility of the population due to vaccination and natural immunization. An survey of an academic setting in Saint Petersburg on almost 4000 samples of students and teachers during the rise in the incidence of COVID-19 (Omicron variant) in January 2022, found that there was a high prevalence of asymptomatic SARS-CoV-2 carriage among the students and teachers of educational establishments located in different parts of the city. On average, the frequency was 20.6% in 14-17-year old adolescents, while in people aged 18 years and older it was 10.1 % [14]. These values seem to be high for a stable endemic situation.

Despite the numerous publications originated in a very short time, several topics remain unanswered, such as the percentage of individuals with natural resistance to infection after successive exposures, the percentage of totally asymptomatic infections according to age range, and the mode of persistence or not of the endemic in a context of a large percentage of vaccinated population. Our work seeks to fill the gaps completing the information about some of these points.

Estimating of asymptomatic SARS-CoV-2 carriage occurrence may contribute to the knowledge of the dynamics and prevention of this infection in relatively closed educational institutions. The estimation of the carriage rate in the study population allowed us to approach the magnitude of the problem and to ponder whether this population of asymptomatic carriers could become a source of contagion in closed spaces such as nosocomial, academic or community setting. The study was designed as an experimental, cross-sectional descriptive study.

2. MATERIALS AND METHODS

The study was conducted in one month period between August and September 2022. By that date, 100% of the cases sequenced for genomic surveillance in all regions of the country corresponded to the Omicron variant [15]. The study was carried out on a population of students of Medical Sciences of the Italian University Institute of Rosario (IUNIR), Argentina. Out of a population of 816 students from a single IUNIR location, 311 students from different career stages were interviewed. They were questioned using a structured survey to verify exclusion criteria and to collect other demographic data of interest. At the time of personal information request and sample extraction, students with fever, persistent cough, lymphadenopathy, headache, antibiotic treatment within 15 days prior to sample collection or sore throat were excluded. Eleven students presented exclusion criteria, so the final population under study was 300 individuals. The subjects included in the study had a sample extracted for RT-PCR for SARS-CoV-2.

Participation was voluntary. The nature and purpose of the study was explained to each participant, and written consent was obtained from those who agreed to participate. Care was taken to maintain the anonymity of the participants by coding the samples. Each participant was given an electronic survey to collect the relevant variables.

2.1 Statistical Analysis

The data were processed by SPSS software (IBM SPSS Statistics V23.0, SPSS Inc., Chicago, Illinois). The minimum sample was calculated using the the Slovin's formula for small populations. Thus, a sample size of 231 individuals was sufficient. Numbers and proportions are presented with 95% confidence intervals.

2.2 Sample Collection and Transport

Extraction of oropharyngeal exudates (OE) was performed with sterile nylon swab by the research team according to a standardized procedure [16] at the IUNIR facilities. Although the OE sample may present lower viral load than the nasopharyngeal sample [17], the analytical sensitivity is similar in both types of samples using RT-PCR [18]. OE was chosen because it minimizes discomfort in the volunteers, also allowing for the detection of carriage of *Streptococcus pyogenes*.

The samples were collected in sterile PBS (phosphate buffered saline) and were transported to the processing center within two hours of being obtained, respecting biosafety measures.

2.3 Sample Processing for Viral Detection

The RNA from samples was extracted using FlashPrep®SARS-CoV2 RNA (Inbio Highway, Tandil, Argentina). SARS-CoV-2 detection was performed by real-time RT-PCR according to the CDC protocol for N1 and N2 gene detection and

using human RNaseP detection as extraction control and a Ct (cycle threshold) <40 as cut-off value [19].

Primers and probes were supplied by IDT (Integrated DNA Technologies, Iowa, USA) and GoTaq® Probe -qPCR (Promega Corp., Madison WI, USA) was used as the reaction mixture. In vitro transcribed RNA (2019-nCoV_N positive control) provided by IDT was used as positive control.

Cycling and detection were performed using MIC thermal cyclers (Bio Molecular Systems, Brisbane, Queensland, Australia). Due to the fact that this cycler allows for the use of a minimum of 10 µl of final reaction volume, the CDC protocol was modified to a final reaction volume of 12.5 µl without loss of sensitivity and with a considerable saving of reagents as we verified during the course of the pandemic.

3. RESULTS

3.1 Demographics

300 individuals of legal age who signed a consent form were swabbed. Samples were collected over a period of one month. Women accounted for 70.3% (n= 211) with an average age of 22 years (range 18-41 years). A very large fraction of the volunteers referred previous SARS-CoV-2 infection (72.7%) confirmed by serology or PCR. All the participants received at least one dose of vaccine with 56.7% received a 3-dose schedule (mostly of different pharmaceutical companies). The data are listed in Table 1. The sampling period was carried out during the epidemiological weeks 35 to 38 (red oval in Figure 1), during this period the viral circulation was low in the Province of Santa Fe.

Table 1. Demographic characteristics of participants and vaccination status

	n	Age (range)	Average Age	Previous confirmed COVID	One single vaccine dose	Two vaccine doses	Three vaccine doses	Four vaccine doses
Female	211 (70.3%)	18-41	22	161 (76.3%)	1	54	126	30
Male	89 (29.4%)	18-34	22	57 (64%)	0	33	44	12
Total	300	18-41	22	218 (72.7%)	1 (0.3%)	87 (29%)	170 (56.7%)	42 (14%)

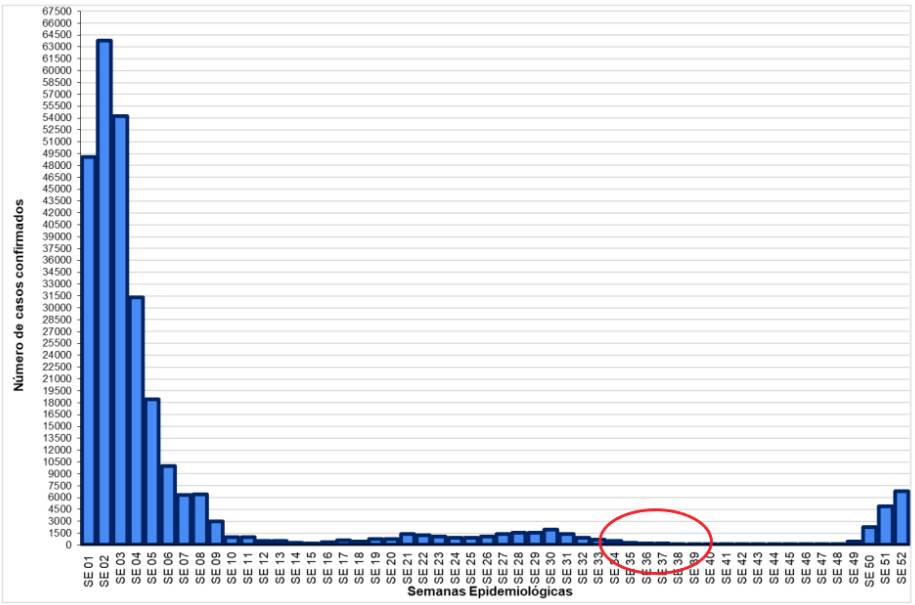

Fig. 1. Distribution of confirmed cases of COVID-19 by epidemiological weeks. Santa Fe Province Year 2022. National Health Surveillance System 2.0 Argentina

Table 2. Data and vaccination history of positive students

ID number	Gender/ Age	Previous COVID	Vaccine doses	1st dose	2nd dose	3rd dose	Ct N1/N2
147	Male/20	2 episodes	2	Pfizer	Pfizer	--	31,11/31.67
244	Female/ 23	1 episode	3	Sinopharm	Sinopharm	Astra Zeneca	24,41/24.98

The patients were swabbed again 15 days after the first extraction, and both were negative

3.2 Viral RNA Detection

Two of the 300 samples tested were positive for SARS-CoV-2. In both cases the two sequences investigated were unequivocally positive with Ct around 31 and 25 (Table 2). The case identified as 147 was classified as presymptomatic because 3 days after the sample was taken it developed symptoms compatible with COVID. Volunteer 244 was the only one classified as an asymptomatic carrier. The prevalence of asymptomatic carriers was 0.33% (95% CI 0.00% –0.95%).

4. DISCUSSION

The SARS-CoV-2 saga is unprecedented in medicine. The spread of the two previous emerging coronaviruses, SARS and MERS, was very limited in time and geographic distribution. For the first time in history we witnessed in real time the pandemic spread of an emerging respiratory virus for which there was no previous immunity. Although previous infections with common cold-producing coronaviruses would confer some protection against severe COVID [20], they would not prevent infection and proof of this was the magnitude of the pandemic. Its appearance and pandemic dissemination were very rapid; also the speed with which the virus was characterized and sequenced allowed the quick design and availability of diagnostic kits [21,22]. The SARS-CoV-2 vaccines with messenger RNA technology were the first of this type to be released to the market [23] and those using an Adenovirus vector were the first to be used massively, since the Ebola vaccine was applied on a limited basis [24]. The worldwide sanitary isolation was also unprecedented and the volume of scientific publications generated in such a short period of time was formidable [25]. The fact of generating scientific information in almost real time has resulted in data that differs greatly depending on the moment of observation (Figure 2). The mortality rate, for example, varies depending on the number of susceptible individuals that decrease over time. The highest initial mortality rates are due to the presence of the maximum possible number of susceptible individuals and patients with comorbidities that increase mortality. The selective pressure of the disease behaves in a darwinian manner, resulting in a population that is enriched in percentage terms in individuals with natural resistance to infection and with individuals that developed acquired immunity either through vaccination or natural infection.

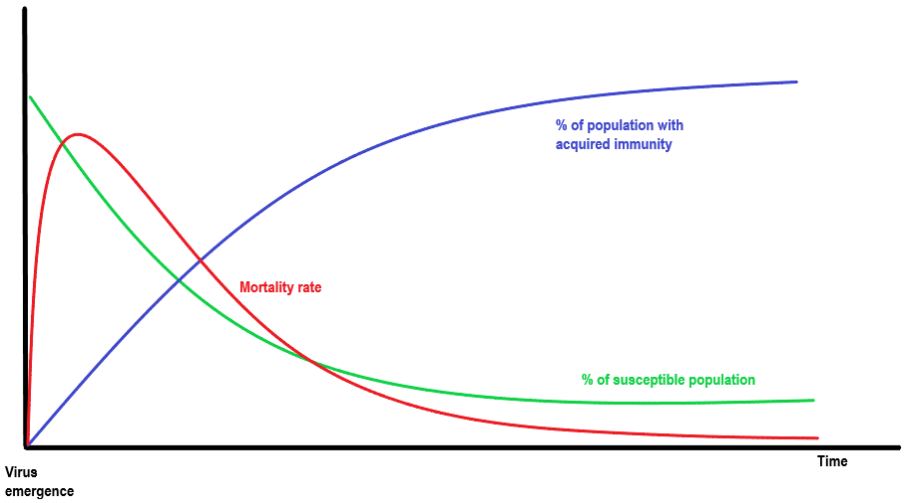

Fig. 2. Variation of epidemiological indices over time

Despite the facts mentioned above, there are still unanswered questions such as the percentage of individuals with innate resistance to infection and the mechanism involved [26], the existence of chronic or long-term carriers as well as the percentage of totally asymptomatic infected persons in each age range. Another aspect to be determined is the role of children in the transmission of the infection, since they are less susceptible to it and to its severe complications [27], with a mortality rate of 0.0003% (range 0.000 to 0.007) in the 0 to 19 years age range [28]. A recent meta-analysis addresses this issue, although in a population with low vaccine coverage [29]. Their findings support the concept that proportions of asymptomatic infection in children and adolescents were higher than adults. A recent advance has been the demonstration of a strong and significant association between a common *HLA* class I allele, *HLA-B*15:01*, with asymptomatic infection with SARS-CoV-2 in unvaccinated individuals [30]. They demonstrated that HLA-B*15:01$^+$ T cells from pre-pandemic samples were reactive to an immunodominant SARS-CoV-2 peptide that shares high sequence similarity with peptides from seasonal coronaviruses.

During the development of the pandemic, it could be seen how often sanitary and isolation recommendations are not based on empirical evidence. An example of this is the use of sodium hypochlorite in footbaths or the disinfection of streets and sidewalks to prevent a respiratory virus. The World Health Organization advised against the use of facemasks in healthy individuals as a method of prevention until March 2020 [31], despite their proven efficacy for more than a century as a method of respiratory isolation.

The obtained rate of asymptomatic portability in our study (0.33%) was surprisingly low. It was similar to that determined for a medical centre in Spain (truly asymptomatic carriage= 0.2%, n= 498) that studied healthcare personnel [32]. However, this study was carried out at the beginning of the pandemic on an unvaccinated population. To date, we have not found research on SARS-CoV-2 carriage in a similar population with equivalent epidemiological conditions in academic search engines.

5. CONCLUSION

The value of asymptomatic carriage obtained in our study (0.33%) was low considering that these are individuals who share an average of 6 hours a day, including lunch in many cases, in closed environments. The whole student population was vaccinated and 72.7% had suffered COVID at the time of sampling, so we can conclude that the risk of contagion in this population and similar ones is extremely low and does not justify the use of masks or social distancing.

Developing studies with this type of design in different populations could lead to the understanding of the dynamics of viral persistence and transmission.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Ethical Committee of the Instituto Universitario Italiano de Rosario with the reference CAI M 03/22.

ACKNOWLEDGEMENTS

The Research Department of I.U.N.I.R and SILAB funded this study.

We express our appreciation to Dr. Hector R. Morbidoni for the critical reading of the manuscript and Dr. Carlos D. De La Vega Elena for statistical advice and critical reading.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Paules CI, Marston HD, Fauci AS. Coronavirus Infections-More than Just the Common Cold. JAMA. 2020 Feb 25;323(8):707–8.
2. Qiang XL, Xu P, Fang G, Liu WB, Kou Z. Using the spike protein feature to predict infection risk and monitor the evolutionary dynamic of coronavirus. Infect Dis Poverty. 2020 Mar 25;9(1):33.
3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet Lond Engl. 2020 Feb 22;395(10224):565–74.
4. Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G. Coronavirus infections:

Epidemiological, clinical and immunological features and hypotheses. Cell Stress. 4(4):66–75.

5. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z, et al. Characteristics of COVID-19 infection in Beijing. J Infect. 2020 Apr;80(4):401–6.
6. Tabata S, Imai K, Kawano S, Ikeda M, Kodama T, Miyoshi K, et al. Clinical characteristics of COVID-19 in 104 people with SARS-CoV-2 infection on the Diamond Princess cruise ship: a retrospective analysis. Lancet Infect Dis. 2020 Sep;20(9):1043–50.
7. World Health Organization. Report of the WHO-China Joint Mission on Coronavirus Disease (COVID-19) (2020); 2019. Available:https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf
8. Wei WE, Li Z, Chiew CJ, Yong SE, Toh MP, Lee VJ. Presymptomatic Transmission of SARS-CoV-2 - Singapore, January 23-March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020 Apr 10;69(14):411–5.
9. Kronbichler A, Kresse D, Yoon S, Lee KH, Effenberger M, Shin JI. Asymptomatic patients as a source of COVID-19 infections: A systematic review and meta-analysis. Int J Infect Dis. 2020 Sep;98:180–6.
10. Sah P, Fitzpatrick MC, Zimmer CF, Abdollahi E, Juden-Kelly L, Moghadas SM, et al. Asymptomatic SARS-CoV-2 infection: A systematic review and meta-analysis. Proc Natl Acad Sci. 2021 Aug 24;118(34):e2109229118.
11. Asadi S, Bouvier N, Wexler AS, Ristenpart WD. The coronavirus pandemic and aerosols: Does COVID-19 transmit via expiratory particles? Aerosol Sci Technol. 2020 Apr 3:1–4.
12. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. JAMA. 2020 Apr 14;323(14):1406–7.
13. Syangtan G, Bista S, Dawadi P, Rayamajhee B, Shrestha LB, Tuladhar R, et al. Asymptomatic SARS-CoV-2 Carriers: A Systematic Review and Meta-Analysis. Front Public Health [Internet]. 2023; 8. Available:https://www.frontiersin.org/articles/10.3389/fpubh.2020.587374
14. Bashketova NS, Семеновна БН, Fridman RK, Кириллович ФР, Kataeva IS, Сергеевна КИ, et al. Analysis of the prevalence and structure of asymptomatic SARS-CoV-2 carriage among adolescents and adults during the COVID-19 epidemic rise in January 2022. Epidemiol Infect Dis Curr Items. 2022 Jan 15;12(1):11–7.
15. Ministerio de Salud, Argentina. Sala de Situación Nacional COVID-19 -Nuevo Coronavirus y otros virus respiratorios SE 39 Año 2022 [Internet]; 2019. Available:https://ciencias.org.ar/user/COVID%202021/ben-620-se-38.pdf
16. Centers for Disease Control and Prevention [Internet]. [cited 2023 Jun 12]. Centers for Disease Control and Infection Prevention. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.; 2020. Available:https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
17. Hitzenbichler F, Bauernfeind S, Salzberger B, Schmidt B, Wenzel JJ. Comparison of Throat Washings, Nasopharyngeal Swabs and Oropharyngeal Swabs for Detection of SARS-CoV-2. Viruses. 2021 Apr;13(4): 653.
18. Calame A, Mazza L, Renzoni A, Kaiser L, Schibler M. Sensitivity of nasopharyngeal, oropharyngeal, and nasal wash specimens for SARS-CoV-2 detection in the setting of sampling device shortage. Eur J Clin Microbiol Infect Dis. 2021 Feb 1;40(2):441–5.
19. Centers for Disease Control and Prevention. CDC 2019-novel coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel services. Centers for Disease Control and Prevention, Atlanta, GA; 2020. Available:https://www.fda.gov/media/134922/download
20. Meyerholz DK, Perlman S. Does common cold coronavirus infection protect against severe SARS-CoV-2 disease? J Clin Invest [Internet]. 2021 Jan 4;131(1). Available:https://www.jci.org/articles/view/144807
21. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8): 727–33.
22. Petherick A. Developing antibody tests for SARS-CoV-2. The Lancet. 2020 Apr 4;395(10230):1101–2.

23. Lamb YN. BNT162b2 mRNA COVID-19 Vaccine: First Approval. Drugs. 2021 Mar 1;81(4):495–501.
24. Gilbert SC. Adenovirus-vectored Ebolavaccines. Expert Rev Vaccines. 2015 Oct 3;14(10):1347–57.
25. Ruiz-Fresneda MA, Jiménez-Contreras E, Ruiz-Fresneda C, Ruiz-Pérez R. Bibliometric Analysis of International Scientific Production on Pharmacologic Treatments for SARS-CoV-2/COVID-19 During 2020. Front Public Health [Internet]. 2022;9. Available:https://www.frontiersin.org/articles/10.3389/fpubh.2021.778203
26. Casanova JL, Su HC, Abel L, Aiuti A, Almuhsen S, Arias AA, et al. A Global Effort to Define the Human Genetics of Protective Immunity to SARS-CoV-2 Infection. Cell. 2020 Jun11; 181(6):1194–9.
27. Brodin P. SARS-CoV-2 infections in children: Understanding diverse outcomes. Immunity. 2022 Feb 8;55(2):201–9.
28. Pezzullo AM, Axfors C, Contopoulos-Ioannidis DG, Apostolatos A, Ioannidis JPA. Age-stratified infection fatality rate of COVID-19 in the non-elderly population. Environ Res. 2023 Jan 1;216:114655.
29. Wang B, Andraweera P, Elliott S, Mohammed H, Lassi Z, Twigger A, Borgas C, Gunasekera S, Ladhani S, Marshall HS. Asymptomatic SARS-CoV-2 Infection by Age: A Global Systematic Review and Meta-analysis. Pediatr Infect Dis J. 2023 Mar 1;42(3):232-239.
30. Augusto DG, Murdolo LD, Chatzileontiadou DSM et al. A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection. Nature. 2023;620:128–136.
31. Médica G. La OMS insiste: Solo enfermos y cuidadores deben usar mascarillas [Internet]. Gaceta Médica; 2020. [Cited 2023 Apr 20]. Available:https://gacetamedica.com/politica/la-oms-insiste-solo-enfermeros-y-cuidadores-deben-usar-mascarillas/
32. Olalla J, Correa AM, Martín-Escalante MD, Hortas ML, Martín-Sendarrubias MJ, Fuentes V, et al. Search for asymptomatic carriers of SARS-CoV-2 in healthcare workers during the pandemic: a Spanish experience. QJM Int J Med. 2020 Nov 1; 113(11):794–8.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/106567