Journal of Hospital Infection 145 (2024) 77–82

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Effects of saline nebulization on SARS-CoV-2 RNA spreading and exhaled bio-aerosol particles in COVID-19 patients

F. Buttini [a], L. Gori [b], F. Morecchiato [b], A. Sorano [b], A. Antonelli [b,c], G.M. Rossolini [b,c], A. Bartoloni [b], J. Mencarini [b], R. Bettini [a], F. Lavorini [b,*]

[a] Food and Drug Department, University of Parma, Parma, Italy
[b] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[c] Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy

ARTICLE INFO

Article history:
Received 16 October 2023
Accepted 16 December 2023
Available online 23 December 2023

Keywords:
Nebulizer
COVID-19
Aerosol
Particles

SUMMARY

Background: Nebulized therapy is the mainstay for treating obstructive airway diseases, but there is heightened concern about the potential risk for SARS-CoV-2 transmission during nebulization in COVID-19 patients.

Aim: To investigate the effects of 0.9% saline nebulization on SARS-CoV-2 RNA spreading in 11 COVID-19 patients (five females, mean age 62.45 ± 9.31 years); also to ascertain whether saline nebulization changed the number of exhaled bio-aerosol particles in six out of the 11 patients.

Methods: Air samples were collected using suction pumps equipped with 0.45 μm PTFE filters and positioned around the patient's bed. Exhaled particles were quantified by using an optical particle counter.

Findings: At baseline (i.e. before nebulization) SARS-CoV-2 was detected more frequently in the pumps close to the patient than in those far away. After saline nebulization, the detection of SARS-CoV-2 in the pumps close to the patient was comparable to that observed at baseline. In the pumps far from the patient, saline nebulization slightly, but not significantly, increased SARS-CoV-2 RNA detection compared to baseline. Overall, no significant changes in the SARS-CoV-2 RNA detection were observed after saline nebulization. At baseline, exhaled particle emission varied among patients, with two of them showing higher emission of particles than the remaining patients. Saline nebulization induced a marked decrease in exhaled particles in the two patients who displayed high emission at baseline, whereas no changes were observed in the remaining patients. Saline nebulization did not significantly change SARS-CoV-2 RNA spreading.

Conclusion: Saline nebulization does not significantly increase SARS-CoV-2 spreading.

* Corresponding author. Address: Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla, Firenze 50135, Italy. Tel.: +39 3486648189.
E-mail address: federico.lavorini@unifi.it (F. Lavorini).

https://doi.org/10.1016/j.jhin.2023.12.008

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has resulted in a worldwide pandemic leading to a major public health crisis. The SARS-CoV-2 virus is transmitted between people through respiratory droplets, bio-aerosols and contact routes [1]. An increased risk of SARS-CoV-2 transmission has been associated with aerosol-generating procedures, including nebulized treatments [2]. Despite the lack of any robust evidence, there is currently a heightened concern regarding the potential risk of transmission of SARS-CoV-2 by patients with COVID-19 undergoing nebulized treatment [3]. While some guidelines have advised against the use of nebulizer treatment unless absolutely necessary, others have recommended its continued use when applicable [4—6]. A recent systematic review concluded that specific evidence that exposure to nebulizer treatment increases transmission of coronaviruses similar to COVID-19 is inconclusive [7]. Aerosols generated by a nebulizer are derived from a medication solution which is not a bio-aerosol — as potentially infectious — as that generated during cough or sneeze [1]. However, nebulizers could generate fugitive emissions during the drug administration, which are added to potentially infectious patient expiration [8]. Fugitive emissions are released from nebulizers and consist of bio-aerosols, which are exhaled by infected individuals during normal tidal breathing or while talking, coughing, or sneezing [9]. Moreover, aerosol delivery devices, including nebulizers, could be theoretically considered as fomites, and, therefore, requiring strict hygiene rules before, during and after use [2]. Interestingly, it has been reported that, in healthy volunteers, nebulization of isotonic saline reduced the number of exhaled bio-aerosol particles by 72% for up to 6 h due to modifications of the surface tension and viscous forces acting on lung-lining fluid [10]. Whether nebulization of isotonic saline aerosols may also reduce exhaled particles in patients affected by viral infection, including SARS-CoV-2, is unknown.

Hence, we investigated the effects of 0.9% saline nebulization on SARS-CoV-2 spreading in patients with COVID-19. An attempt was also made to ascertain whether saline nebulization changed the exhaled bio-aerosol particles in these patients.

Methods

This was a cross-sectional study performed from December 2021 to May 2022 on adult patients of both genders admitted to the Careggi University Hospital (Florence, Italy) for COVID-19. SARS-CoV-2 infection was tested on the same day as an air-sample collection with the reverse transcription—real-time polymerase chain reaction test (RT—PCR) from nasopharyngeal swab (NPS) in UTM® medium (Copan Group, Brescia, Italy); RNA extraction and SARS-CoV-2 quantitative evaluation from 200 μL of NPS was performed with the ELITe MGB® Kit on the ELITe InGenius® automated system (ELITechGroup SAS, Puteaux, France). Patients requiring mechanical ventilation, those who were breastfeeding or pregnant, and those who had a significant comorbidity making them unsuitable for participation in the opinion of the investigator were excluded. All procedures complied with the Helsinki Declaration. The study was approved by the local Institutional Review Board (20339_spe); a written informed consent was obtained from participants after a detailed explanation of the purposes of the study. Patients were admitted in a single occupancy, negative-pressure room with four to eight air exchanges per hour. Efforts were made to minimize any non-study activity within the patient's room during the viral collection as well as the entry of any additional personnel during this period. At all times, healthcare staff and clinical trial team members entering patients' rooms were required to wear personal protective equipment including an FFP2/N95 or higher-level mask, eye protection, gloves and gown. Sampling of the room air was carried out according to the ISPE guidelines for the particle environmental contamination using five suction XR5000 pumps (SKC Inc., Eighty-Four, PA, USA) equipped with a 0.45 μm PTFE filter [11]. Each pump ran at 3 L/min and was positioned around the patient's bed at different heights and distances (Figure 1) in accordance with the World Health Organization suggestions for sampling the airflow in the room of a COVID-19 patient [12]. In more detail, the distance from the patient's head and the pumps ranged from 30 to 300 cm (Figure 1). The distances between patients and the sampling pumps were consistent across all hospital rooms. In each patient, control (i.e. prior nebulization) air-sample collections lasted for 3 h. During the air-sampling period, patients were free to talk, cough, and sneeze; no attempt was made to objectively assess the number of coughs and/or sneezes. After the 3 h of continuous sampling, filters were removed, sealed, and examined for SARS-CoV-2 RNA detection. The latter was qualitatively obtained by using the Allplex™ SARS-CoV-2 assay (Seegene, Inc., Seoul, Korea). A quantitative analysis of SARS-CoV-2 RNA was also performed with SARS-CoV-2 ELITe MGB® Kit on ELITe InGenius® (ELITechGroup) and Droplet digital PCR assays (ddPCR). Samples were considered as positive for SARS-CoV-2 when a signal was detected by at least one of the molecular assays tested in order to increase sensitivity. Further details on the methodology of SARS-CoV-2 detection are reported in the Supplementary Appendix.

After the air-sampling control, each patient, wearing a nose clip, was asked to breathe normally through a mouthpiece connected to a sampling T adapter, with one end of the T adapter connected to a six-channel optical particle counter (Ultimate 100, Climet, West Colton Avenue, Redlands, CA, USA) to measure the number and size of expired particles. Each channel on the optical counter tabulates particle counts within a size-selective range for a total of six bins: >0.5, 0.5—1.0, 1—3, 3—5, 5—10, and >10 μm. The other end of the T-adapter was connected to a Delbag—Luftfilter air filter (Copular CKL Macropur-F Acelan; GEA, Berlin, Germany) for removal of any airborne particulates from the inhaled ambient air stream. After a 2 min session of breathing through the Delbag—Luftfilter air filter to remove ambient particles, patients breathed through the system for two 1 min sessions and the optical particle counter tabulated average particle concentration and size in the exhaled air. Subsequently, patients inhaled 5 mL of 0.9% saline delivered by a Pari LC Plus Jet nebulizer (Pari, Starnberg, Germany) connected to a compressed air source. Nebulization time lasted about 5 min. For each patient, air samples were collected with the same procedure described above during nebulization and for 3 h afterwards; measurement of average particle concentration and size in the exhaled air were also reassessed 15 and 60 min after nebulization as described above. Cleaning and disinfection of the optical particle counter was carried out at the

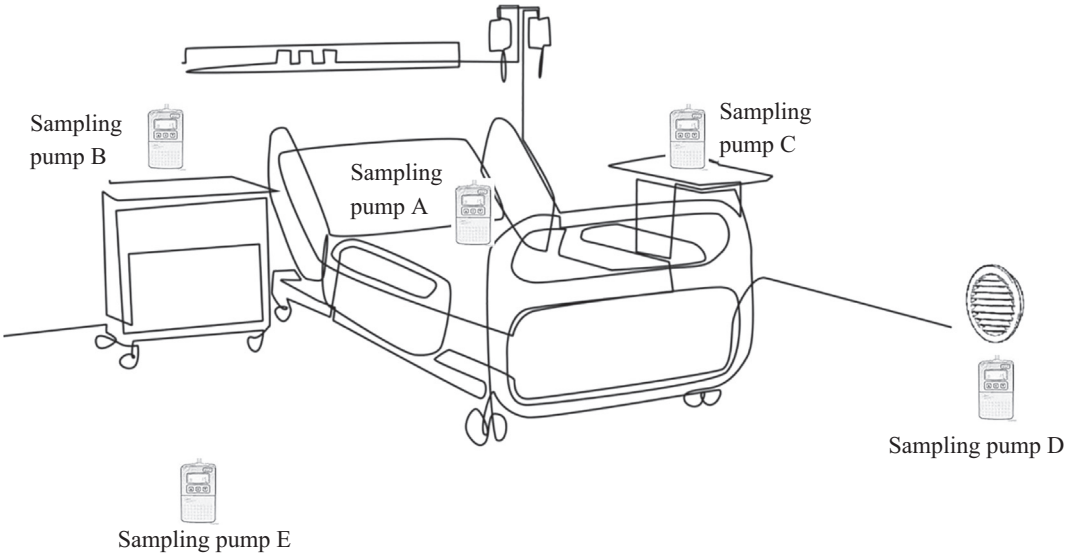

Figure 1. Hospital room sampling layout. Sampling pump A was positioned at a height 140 ± 10 cm from the ground and 30 ± 5 cm from the patient's head; sampling pumps B and C were positioned on the right and left side of the patients, at a height of 120 ± 10 cm from the ground and a distance of 100 ± 10 cm from the patient's head; sampling pump D was positioned at a height 200 ± 10 cm from the ground and at a distance of 300 ± 10 cm from the patient's head; sampling pump E was positioned near the ventilation grille, at a height of 120 ± 10 cm from the ground, and at a distance of 300 ± 10 cm from the patient's head.

end of each investigation with vaporized hydrogen peroxide as suggested by the manufacturer's instructions for use.

Data analysis

Based on the environmental sampling scheme employed in COVID-19 clinical studies, a sample size of ≥10 patients was deemed appropriate to assess the impact of nebulization on SARS-CoV-2 spreading [13,14]. Continuous variables are reported as mean ± standard deviation (SD) or as median and 95% confidence interval (CI) for non-normal distributions and they were compared by parametric or non-parametric *t*-test. Categorical variables are reported as counts and percentages and were compared among groups with the χ^2-test (or Fisher's exact test when appropriate). A comparison between the number of samples positive for SARS-CoV-2 before and after nebulization was performed by means of the Fisher's exact test. Changes in the particle size distribution after saline nebulization were assessed by non-parametric analysis of variance for repeated measures. All statistical tests were two-tailed and statistical significance was assumed for $P<0.05$.

Results

Sixteen patients were enrolled (five females; mean age: 60.15 ± 18.05 years) with an RT—PCR positive for SARS-CoV-2 RNA and viral loads in NPS ranging from 2×10^2 to 2×10^8 copies/mL (mean 7×10^7 copies/mL). Duration of symptoms

Table I
Demography and clinical characteristics of the 11 COVID-19 patients

Patient no.	Sex	Age (years)	BMI (kg/m^2)	NPS viral copies/mL	Days after symptom onset	Vaccine status	Oxygen flow (L/min)
1	Female	58	25.41	2×10^6	3	No	2.0
2	Male	51	27.12	2×10^2	2	No	4.0
3	Male	70	22.32	9×10^5	2	No	3.0
4	Female	69	27.15	9×10^5	4	Yes	2.0
5	Female	50	25.78	5×10^6	2	Yes	3.0
6	Male	48	28.15	5×10^7	2	Yes	4.0
7	Male	74	23.12	2×10^8	3	Yes	1.0
8	Female	71	25.55	2×10^8	3	Yes	5.0
9	Male	69	29.50	6×10^5	4	Yes	3.0
10	Female	64	30.14	2×10^6	3	Yes	4.0
11	Male	63	28.12	2×10^8	5	Yes	3.0
Mean		62.45	26.58		3.00		
SD		9.31	2.45		1.00		

BMI, body mass index; NPS, nasopharyngeal swab.

before hospital admission was similar among patients. At hospital admission, all patients required supplementary oxygen and seven of them (63%) were not vaccinated. All patients reported respiratory symptoms such as dyspnoea, dry cough, nasal obstruction, and sneezing. Five out of the 16 patients dropped out due to worsening of the disease whereas the remaining 11 patients (Table I) completed the pre- and post-nebulization runs. Thus, the total number of air samples evaluable for SARS-CoV-2 RNA detection was 110, equally divided between pre- and post-nebulization periods. At baseline, i.e. prior to nebulization, nine out of the 11 patients had air sample filters positive in at least one of the five selected sites (Table II). Air sample filters from the remaining two patients were always negative for SARS-CoV-2 RNA detection. Although not formally assessed, the NPS was unrelated to SARS-CoV-2 RNA dissemination in the air. In patients who had positive air filters prior to the saline nebulization, SARS-CoV-2 RNA was more frequently detected in the filters close to the patient (pumps A—C) (8/33, 24%) than in those further away (pumps D and E) (2/22, 9%). After saline nebulization, six out of the 11 patients displayed air sample filters positive for SARS-CoV-2 in four (pumps A, C—E) out of the five pumps for a total of 12 positive filters (12/55, 21%). More specifically, in the pump filters positioned close to the patient (pumps A—C), detection of SARS-CoV-2 RNA was similar or lower after saline nebulization compared to baseline (Table II). In contrast, in the pump filters positioned far from the patient (pumps D and E) saline nebulization slightly, but not significantly (Fisher's exact test), increased SARS-CoV-2 RNA detection compared to baseline (Table II). Thus, detection of SARS-CoV-2 RNA was unchanged after saline nebulization. Overlapping results were obtained when using quantitative analyses for SARS-CoV-2 RNA detection. In a subgroup (N=6) of patients, the number and size distribution of exhaled particles before and after nebulization were also measured. At baseline, a large variability was found among patients in the exhaled particles emission, with two patients (nos. 1 and 5) showing higher emission of exhaled particles than the remaining patients (Figure 2). Saline nebulization induced a marked decrease in exhaled bio-aerosol in the two patients who displayed high emission at baseline, whereas no changes were observed in the remaining patients (Figure 2). Overall, the median number of exhaled bio-aerosol particles measured before saline nebulization (59.50; 95% CI: −70.49 to 490.8) did not significantly differ from that obtained 15 min (70.00; −8.38 to 242.1) and 60 min (68.50; 9.04 to 156.7) after saline nebulization.

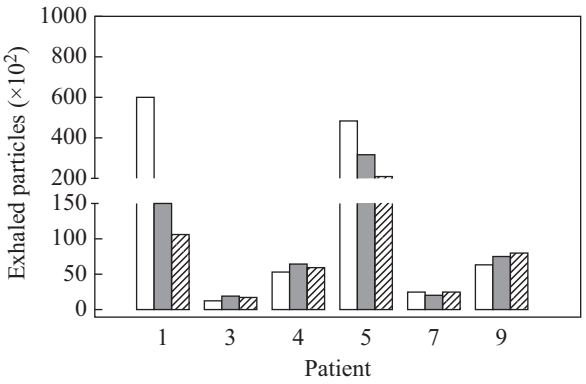

Figure 2. Total number of exhaled particles measured before (empty columns), 15 min (grey columns), and 60 min (hatched columns) after saline nebulization in six COVID-19 patients.

The size distribution of exhaled particles is reported in Figure 3. At baseline, most of the exhaled particles had sizes between 1 and 0.5 μm. The particle size distribution shifted toward the range 5—0.5 μm 15 min after saline nebulization, while it turned out to overlap the baseline distribution after 60 min (Figure 3). However, in all cases, these changes did not reach statistical significance.

Discussion

This study investigated SARS-CoV-2 RNA spreading in a negative-pressure hospital room before and after saline nebulization. At baseline, SARS-CoV-2 RNA was found in 10 out of

Table II
Presence (+) or absence (−) of SARS-CoV-2 RNA in the filters of the five pumps before (pre) and after (post) saline nebulization in the 11 COVID-19 patients revealed by at least one molecular assay tested

Patient no.	Pump A		Pump B		Pump C		Pump D		Pump E	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	−	−	+ [<250] (19)	−	+ (10)	−	−	−	+[a]	+ (21)
2	−	−	−	−	−	−	−	−	−	+[a]
3	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	+ [<250]	−	−	−	−	−
6	+ (42)	+[a]	−	−	−	+ (8)	−	+ (21)	−	−
7	+ [<250] (10)	+ (11)	−	−	−	−	−	+[a]		+ [<250]
8	−	+ [<250]	−	−	+ [<250] (22)	−	+ [<250]	−	−	−
9	+ [<250 (7)	−	−	−	−	−	−	+[a]	−	−
10	−	−	−	−	−	−	−	+[a]	−	+ [<250]
11	−	−	−	−	+[a]	−	−	−	−	−

RT—PCR, reverse transcription—polymerase chain reaction.
SARS-CoV-2 RNA copies/mL for positive sample are indicated in square brackets for SARS-CoV-2 ELITe MGB® Kit (ELITechGroup SAS) and curved parentheses for droplet digital PCR.
[a] SARS-CoV-2 RNA was detected only with Allplex™ SARS-CoV-2 assay (Seegene, Inc.) qualitative RT—PCR.

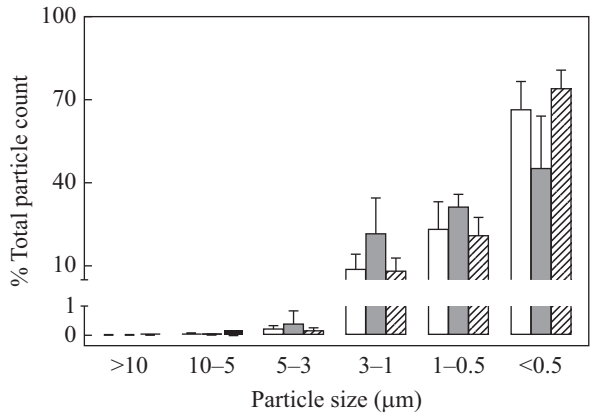

Figure 3. Distribution of exhaled particle sizes, expressed as percentages of the total number of exhaled particles, obtained at baseline (empty columns), 15 min (grey columns), and 60 min (hatched columns) after saline nebulization in six COVID 19 patients. Data are means ± standard deviation.

55 (18%) air samples irrespective of the sampling areas. Furthermore, qualitative and quantitative analyses revealed that saline nebulization did not significantly change SARS-CoV-2 RNA spreading.

It is worth noting that, before nebulization, detection of SARS-CoV-2 RNA differed among the different air-sampling areas, with SARS-CoV-2 RNA more frequently detected in the air samples closer to the patient than those further away. In contrast, after saline nebulization, detectable levels of SARS-CoV-2 RNA were mainly found in the air samples far from the patient, particularly near the ventilation air-extract grille. Thus, we can conclude that saline nebulization reduced air contamination by SARS-CoV-2 near the patient and that this effect was reduced when moving away from the patient. The reasons for this finding are not immediately clear. However, we can speculate that, as regards the influenza virus which has viral particle sizes similar to SARS-CoV-2, significant amounts of SARS-CoV-2 RNA were trapped in aerosol particles small (<5 μm) enough to be transported by the air over a certain distance [15]. Accordingly, Gohli *et al.* recently found that, in aerosol particles sized between 3 and 5 μm, the concentration of SARS-CoV-2 RNA was higher during nebulization than prior nebulization [16]. Therefore, the lack of significant difference in SARS-CoV-2-spreading after nebulization could be related to differences in bio-aerosol emission, with smaller or larger particles travelling in the air for a greater or shorter distance, respectively. However, as reported in Figure 3, 60 min after nebulization the particle size distribution was similar to that observed at baseline (i.e. prior nebulization), thus justifying the absence of differences in the pump sampling observed 3 h after nebulization.

We attempted to assess changes in exhaled bio-aerosol particles induced by saline nebulization. In agreement with Edwards *et al.*, we found a large variability among patients in the total number of exhaled bio-aerosol particles with two out of the six patients studied showing a high number of exhaled particles [10]. In these patients, saline nebulization markedly reduced the number of exhaled bio-aerosol particles; interestingly, in the patients emitting a higher number of exhaled particles, saline nebulization also induced a reduction in SARS-CoV-2 RNA detection in all air-sampling areas. Further larger studies are needed to clarify this finding.

This study has some limitations. SARS-CoV-2 RNA was detected by RT—PCR and ddPCR, which are highly sensitive but do not differentiate infectious from non-infectious viral particles. Furthermore, the data were based on two sampling periods (pre and post nebulization) per patient and did not consider potential within-patient variability. At the time of the study, the SARS-CoV-2 Omicron variant was the most prevalent in Italy, and it is believed to be the main variant of the enrolled patients [17,18]. However, the SARS-CoV-2 variant was not assessed in this study, and, therefore, present findings may not be generalizable across variants which may vary in transmissibility. The results of this study were obtained in a small sample of patients and, therefore, warrant confirmation in larger trials. However, it is worth noting that studies aimed at investigating environmental contamination caused by SARS-CoV-2 involved a group of patients similar to the one in this study [13,14]. More importantly, this investigation is the largest real-life study involving COVID-19 patients to assess the impact of nebulization on virus dissemination. Finally, we studied patients with mild COVID-19 admitted in pressure-negative hospital rooms; thus, the results obtained here cannot be extrapolated from situations differing from those employed in this study.

Case series raise concern of transmission risk, and simulation studies demonstrate droplet dispersion with virus recovery, but specific evidence that exposure to nebulizer treatments increases SARS-CoV-2 transmission is inconclusive [7]. However, in this study, saline nebulization did not lead to increased dispersion of SARS-CoV-2 RNA, with low environmental exposure observed regardless of the use of the nebulized therapy. These findings, along with the recent demonstration of the absence of nebulizer contamination by SARS-CoV-2 in hospitalized patients, are reassuring and, therefore, there is no compelling reason to withhold nebulized therapy in COVID-19 patients who may benefit from this treatment method [19].

Acknowledgements

The authors are grateful to Mrs S. Cadby Berardi for the English revision of the manuscript.

Author contributions

F.L.: study conceptualization and coordination, data collection and analysis, interpretation, drafting and revision of the manuscript; F.B.: study conceptualization, data collection and analysis, interpretation, drafting and revision of the manuscript; L.G.: patient enrolment coordination, data collection and analysis, interpretation, revision of the manuscript; F.M.: study conceptualization, laboratory data collection and analysis, interpretation, drafting and revision of the manuscript; A.S.: patients enrolment, data collection and statistical analysis, interpretation, revision of the manuscript; A.A.: study conceptualization, laboratory data collection and analysis, interpretation, drafting and revision of the manuscript; G.M.R.: study conceptualization, laboratory data collection and analysis, interpretation, revision of the manuscript; A.B.: study conceptualization and coordination, data collection and analysis, interpretation,

revision of the manuscript; J.M.: patient enrolment, data collection and analysis, interpretation, revision of the manuscript; R.B.: study conceptualization and coordination, interpretation, drafting and revision of the manuscript. F.L., F.B., and F.M. have verified the underlying data. All authors had full access to all the data in the study and accept responsibility for submitting the paper for publication. Both F.L. and F.B. were responsible for the manuscript preparation.

Conflict of interest statement

F.L. received research grants from Chiesi Farmaceutici, Parma, Italy. The other authors have no potential conflict to declare.

Funding sources

This work was supported by an unconditional grant from Chiesi Farmaceutici, Parma, Italy. Chiesi Farmaceutici had no role in the study design, data collection, analysis and interpretation, or in writing the report or in the decision to submit the paper. All authors had access to the dataset and agreed to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2023.12.008.

References

[1] Dhand R, Li J. Coughs and sneezes: their role in transmission of respiratory viral infections, including SARS-CoV-2. Am J Respir Crit Care Med 2020;202:651—9.

[2] Lavorini F, Usmani OS, Dhand R. Aerosol delivery systems for treating obstructive airway diseases during the SARS-CoV-2 pandemic. Intern Emerg Med 2021;16:2035—9.

[3] Amirav I. Aerosol treatments during COVID19 pandemic: a personal journey. J Aer Med Pulm Drug Deliv 2020;33:1—24.

[4] Global Initiative for Asthma. GINA Report. Global strategy for asthma management and prevention. 2020. Available at: https://www.ginasthma.com [last accessed January 2024].

[5] Australian National Asthma Council. Managing asthma during the COVID-19 (SARS-CoV-2) pandemic. COVID-19. Available at: https://www.Asthmahandbook.org [last accessed January 2024].

[6] National Institute for Health and Care Excellence. COVID-19 rapid guideline: severe asthma (NICE guideline [NG166]). 2020. Available at: https://www.nice.org.uk/guidance/ng166 [last accessed January 2024].

[7] Goldstein KM, Kamrouz Ghadimi K, Mystakelis H, Kong Y, Meng T, Cantrell S, et al. Risk of transmitting coronavirus disease 2019 during nebulizer treatment: a systematic review. J Aerosol Med Pulm Drug Deliv 2021;34(3).

[8] Harding H, Broom B, Broom J. Aerosol generating procedures and infective risk to healthcare workers: SARS-CoV-2 the limits of evidence. J Hosp Infect 2020;105:717—25.

[9] Biney I, Ari A, Barjaktarevic IZ, Carlin B, Christiani DC, Cochran L, et al. Guidance on mitigating the risk of transmitting respiratory infections during nebulization by the COPD Foundation Nebulizer Consortium. Chest 2023 Nov 15. https://doi.org/10.1016/j.chest.2023.11.013 [online ahead of print].

[10] Edwards DA, Man JC, Brand P, Katstra JP, Sommerer K, Stone HA, et al. Inhaling to mitigate exhaled bioaerosols. Proc Natl Acad Sci USA 2004;101:17383—8.

[11] International Society for Pharmaceutical Engineering. Good Practice Guide: assessing the particulate containment performance of pharmaceutical equipment, 2nd edn. Available at: Ispe.org/publications/guidance-documents/assessing-particulate-containment-performance [last accessed January 2024].

[12] World Health Organization. Surface sampling of coronavirus disease (COVID-19): a practical "how to" protocol for health care and public health professionals. Available at: https://iris.who.int/bitstream/handle/10665/331058/WHO-2019-nCoV-Environment_protocol-2020.1-eng.pdf?isAllowed=y&sequence=1 [last accessed January 2024].

[13] Santarpia JL, Rivera DN, Herrera VL, Morwitzer MJ, Creager HM, Santarpia GW, et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. Sci Rep 2020;10:12732.

[14] Chia PY, Coleman KK, Tan YK, Ong SWX, Gum M, Lau SK, et al. Singapore novel coronavirus outbreak research, detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat Commun 2020;11:2800.

[15] Scheuch G. Breathing is enough: for the spread of influenza virus and SARS-CoV-2 by breathing only. J Aerosol Med Pulm Drug Deliv 2020;33:230—4.

[16] Gohli J, Brantsæter AB, Bøifot KO, Grub C, Granerud BK, Holter JC, et al. SARS-CoV-2 in the air surrounding patients during nebulizer therapy. Can J Infect Dis Med Microbiol 2022;2022:9297974.

[17] Stefanelli P, Trentini F, Petrone D, Mammone A, Ambrosio L, Manica M, et al. Genomic SARS-CoV-2 National Surveillance Working Group; Italian Integrated Surveillance of COVID-19 Study Group. Tracking the progressive spread of the SARS-CoV-2 Omicron variant in Italy, December 2021 to January 2022. Euro Surveill 2022;27:2200125.

[18] Modenese A. Correlations between SARS-CoV-2 infections and the number of COVID-19 vaccine doses administered in three Italian provinces. Healthcare (Basel) 2023;11:358.

[19] Thibon C. Nebulization and COVID-19: is the risk of spread actual? Respir Med 2022;197:106854.