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Low Risk of Severe Acute Respiratory Syndrome Coronavirus 2 Transmission by Fomites: A Clinical Observational Study in Highly Infectious Coronavirus Disease 2019 Patients

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Background. The contribution of droplet-contaminated surfaces for virus transmission has been discussed controversially in the context of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic. More importantly, the risk of fomite-based transmission has not been systematically addressed. Therefore, the aim of this study was to evaluate whether confirmed hospitalized coronavirus disease 2019 (COVID-19) patients can contaminate stainless steel carriers by coughing or intensive moistening with saliva and to assess the risk of SARS-CoV-2 transmission upon detection of viral loads and infectious virus in cell culture.

Methods. We initiated a single-center observational study including 15 COVID-19 patients with a high baseline viral load (cycle threshold value $\leq$25). We documented clinical and laboratory parameters and used patient samples to perform virus culture, quantitative polymerase chain reaction, and virus sequencing.

Results. Nasopharyngeal and oropharyngeal swabs of all patients were positive for viral ribonucleic acid on the day of the study. Infectious SARS-CoV-2 could be isolated from 6 patient swabs (46.2%). After coughing, no infectious virus could be recovered, however, intensive moistening with saliva resulted in successful viral recovery from steel carriers of 5 patients (38.5%).

Conclusions. Transmission of infectious SARS-CoV-2 via fomites is possible upon extensive moistening, but it is unlikely to occur in real-life scenarios and from droplet-contaminated fomites.

Keywords. SARS-CoV-2; COVID-19; fomite transmission; surface stability

The emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of the coronavirus disease-19 (COVID-19), has raised the general awareness towards different hygiene and prevention measures to limit viral spread. Although SARS-CoV-2 is mainly transmitted via respiratory droplets and aerosols exhaled from infected individuals (eg, upon breathing, speaking, coughing or sneezing [1]), droplet-contaminated surfaces (fomites) have also been widely perceived as another potential route of transmission. In particular, different studies reported that SARS-CoV-2 can persist on inanimate surfaces for days under controlled laboratory conditions [2–4], and genomic material of SARS-CoV-2 has been detected on diverse surfaces and materials in hospital, private, and public settings [5]. As a result, a clinically significant risk of SARS-CoV-2 transmission by fomites has been assumed, and extensive hand hygiene and disinfection procedures have been initiated early during the pandemic worldwide. Although recent studies suggest a low risk of viral transmission by fomites for most instances [1, 6], it is still considered possible given a timely order of events (eg, direct contamination of a surface by an infected individual followed by timely skin contact by another individual and direct contact towards susceptible mucosae) [7]. However, most efforts to study surface transmission of SARS-CoV-2 have either focused on the detection of viral ribonucleic acid (RNA) via quantitative reverse-transcription polymerase chain reaction (RT-qPCR) rather than direct detection of infectious viral particles and/or used laboratory-grown viruses that do not recapitulate the specific infectivity of patient-derived SARS-CoV-2 particles.

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Therefore, these findings do not necessarily allow researchers to adequately estimate the potential of SARS-CoV-2 transmission from directly contaminated surfaces.

To examine the risk of transmission by surfaces directly after contamination by individuals infected with SARS-CoV-2, we performed a clinical observational study, including hospitalized patients with high viral loads (cycle threshold [CT] ≤ 25, up to 2.03×10^9 RNA copies). The aim of this study was to evaluate whether confirmed hospitalized COVID-19 patients can contaminate stainless steel carriers by coughing or intensive moistening with saliva and to assess the risk of SARS-CoV-2 transmission upon detection of viral loads and infectious virus in cell culture.

METHODS

Study Cohort

Hospitalized patients (age > 18 years) treated at St. Josef-Hospital Bochum, Germany, with confirmed SARS-CoV-2 infection with a high virus load (RT-PCR from combined nasopharyngeal and oropharyngeal swab [swab] with a CT $\leq$25 on admission) were included in this study. The initial viral load was determined using Allplex 2019-nCoV Assay (Seegene Inc., Seoul, Republic of Korea) targeting 3 SARS-CoV-2-specific genes (E gene, RdRP gene, and N gene) with a sensitivity of 100 copies per run. Exclusion criteria were acute myocardial infarction, current need of ventilation support systems (eg, high flow- or noninvasive ventilation), current treatment in an intensive care unit, evidence of drug or alcohol abuse, acute psychiatric disorders, and any clinical or mental disorder that might deteriorate the patient's condition during the standardized procedure of sampling (such as dysphagia), as per investigator's judgment. Collected clinical data included medical history, current daily medication, laboratory results, blood gas analysis, and results of x-rays or computed tomography (to define the "clinical classification of COVID-19-infection", we followed the recommendation of the World Health Organization [WHO] [8] and the Robert Koch Institute [RKI] [9]).

Study Design

After written informed consent, 2 combined nasopharyngeal and oropharyngeal swabs were collected from each patient. Then, patients were asked to forcefully cough 2 times on a predefined surface area containing 9 standardized steel carriers, each with a 1-centimeter diameter ("cough"), using a specially designed tripod with a defined distance of 15 centimeters (Supplementary Appendix Figure 1). In addition, patients were asked to moisten 9 steel carriers with saliva for 10 seconds within their mouth ("moisten"). After defined time points at room temperature (1 minutes, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 90 minutes, 120 minutes and 240 minutes), the steel carriers were placed in containers containing 2 mL cold Dulbecco's modified Eagle's medium (DMEM complete, supplemented with 10% [v/v] fetal calf serum, 1% nonessential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine) and transported on ice to the biosafety level 3 laboratory of the Ruhr-University Bochum. The study was conducted between November 2020 and April 2021.

Virus Culture

VeroE6 cells were seeded at 3×10^5 cells/well in a 6-well cell culture plate and incubated for at least 4 hours at 37 °C and 5% CO_2. Hereafter, the medium was replaced with 1.8 mL patient swabs, cough samples, or moisten samples, and 2.5 µg/mL amphotericin B was added. Over a maximum period of 10 days, cells were monitored daily for the appearance of a cytopathic effect (CPE), indicating productive virus infection. Upon visible CPE, cells were harvested for RT-qPCR, and the supernatant (SN) was collected for viral titration and RT-qPCR. Viral titers in the SN were quantified by endpoint-dilution and the 50% tissue culture infective dose ($TCID_{50}$/mL), calculated according to Spearman and Kärber [10].

Reverse-Transcription Quantitative Polymerase Chain Reaction

Severe acute respiratory syndrome coronavirus 2 RNA was isolated from the supernatant using AVL buffer and the QIAamp Viral RNA Kit (QIAGEN, www.qiagen.com) according to the manufacturer's instructions. The RNA was directly subjected to one-step RT-qPCR running a GoTaq Probe 1-Step RT-qPCR System (Promega, www.promega.com). Total RNA was purified from VeroE6 cells using the RNeasy Mini Kit (QIAGEN). Subsequently, 500 ng of total RNA were reverse transcribed using the PrimeScript RT Master Mix (Takara, www.takarabio.com) and subjected to 2-step RT-qPCR running a GoTaq Probe 2-Step RT-qPCR System (Promega). The RT-qPCR was performed as described previously [11] using a light cycler LC480 to quantify the M-Gene abundance.

Data Analysis and Sample Size

Clinical patient parameters are expressed as mean $\pm$ standard deviation or n (% of total). Results are expressed as means ($\pm$standard error of the mean). Clinical characteristics were screened for correlations using Spearman's correlation coefficient. Statistical significance was defined as $\alpha = 0.05$. Statistical analysis was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA). Sample size calculation was performed using G*Power Version 3.1.9.6 for windows [12].

Legal and Ethical Considerations

The study was conducted according to the revised principles of the Declaration of Helsinki and was approved by the ethics committee of the Ruhr-University Bochum (registration number 20-7065) in November 2020. All patients gave written informed consent.

Table 1. Baseline Parameters Including Laboratory Findings of the Study Group[a]

Parameter	Unit	Normal Range	Result
Participants	n	n/a	15
Age	Years	n/a	70.5 (±12.3)
Male/female (%female)	n	n/a	10/5 (33.3%)
BMI	kg/m²		28.8 (±6.0)
Arterial hypertension	n	n/a	10 (66.7%)
ACE inhibitors	n	n/a	3 (20%)
Dyslipidemia	n	n/a	5 (33.3%)
Diabetes mellitus	n	n/a	5 (33.3%)
Currently smoking	n	n/a	8 (53.3%)
Family history of CVD	n	n/a	4 (26.7%)
Immunosuppression	n	n/a	5 (33.3%)
Active malignancy	n	n/a	4 (26.7%)
Chronic pulmonary disease	n	n/a	7 (46.7%)
Chronic renal disease	n	n/a	3 (20%)
Leucocytes	/µL	4600–9500	6182.7 (±3560.0)
Hemoglobin	g/dL	14.0–18.0	11.5 (±2.6)
Thrombocytes	/µl	150 000–400 000	175 933.3 (±64 077.5)
D-dimers	µg/mL	<0.5	1.3 (±0.9)
Lactate dehydrogenase	U/L	135–225	250 (±63)
eGFR	mL/min	>90	63.4 (±26.4)
Procalcitonin	ng/mL	<0.5	33.5 (±124.9)
C-reactive protein	mg/L	<5.0	41.8 (±38.9)
pH		7.35–7.45	7.4 (±0.1)
Bicarbonate	mmol/L	22–26	26.1 (±4.3)
Peripheral oxygen saturation	%	≥95	93.4 (±7.4)
Air temperature	°C	n/a	5.5 (±5.5)
Air humidity	%	n/a	72.8 (±18.7)

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; n/a, not applicable.

[a]Data are presented as n (% of total) or mean (±standard deviation). Risk factors for severe coronavirus disease 2019 were defined as age >50 years, male sex, smokers, adiposity (BMI >30), Down syndrome, history of cardiovascular diseases, chronic lung disease, chronic renal disease, psychiatric diseases, diabetes mellitus, malignant or hemic diseases, or immunodeficiency [14]. Estimated GFR was estimated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [15]. Peripheral oxygen saturation was determined on a finger, using a pulse oximeter.

Table 2. Viral Load Before Sample Acquisition Using Naso-Oropharyngeal Swabs

Patient	Initial CT Values (*E-Gene/S-Gene/RdRP-Gene/N-Gene*; VOC-Analyses[a])	Deep Sequencing	Mutations Identified in Spike Domains
P1	13/-/17/15	B.1.389	**D614G**, T723I
P2	14/-/16/17	B.1.1.70	**D614G**
P3	13/-/14/15	B.1.1.70	**D614G**
P4	16/-/17/20	B.1.1.70	**D614G**
P5	12/-/13/14	B.1.221	S98F, **D614G**
P6	17/-/18/16	B.1.177.7	A222V, **D614G**
P7	21/-/22/19	B.1.177	A222V, **D614G**, A1020V
P8	16/-/16/18	B.1.221	S98F, L141LF, **D614G**
P9	-/16/16/14	B.1.177	A222V, **D614G**
P10	-/12/13/13	B.1.1.153	**D614G**
P11	-/24/25/23; VOC B1.351[a]	B.1.351	H69Y, **D80A**, **D215G**, del242-244, **K417KT**, **E484EK**, **N501NY**, **D614G**, A701V
P12	-/24/23/32; VOC B1.1.7[a]	-[b]	-[b]
P13	-/17/16/25; VOC B1.1.7[a]	B.1.1.7	**del144, N501NY, A570D, D614G, P681H,** A694AS, **T716I, S982A, D1118H**
P14	-/13/12/21; VOC B1.1.7[a]	B.1.1.7	**del69-70**, S98F, D138DH, H245Y, **N501NY**, **A570D**, **D614G, P681H**, A694AS, **T716I, S982A, D1118DH**
P15	-/21/20/29; VOC B1.1.7[a]	B.1.1.7	**del69-70, del144, N501Y, A570D, D614G, P681H**, A694AS, **T716I, S982A, D1118H**

Abbreviations: CT, cycle threshold resulting from RT-PCR performed at St. Josef-Hospital Bochum; VOC, variants of concern.

NOTE: Data are presented as individual data. Lineage of deep sequencing is presented according to the pangolin tool [13]. Bold = characteristic mutations associated with VOC according to Robert Koch Institute [17].

[a]VOC analyses started in the midst of the study period in February 2020 and was performed via melting curve analysis.

[b]No sufficient material available.

Sequencing and Strain Assignment

The RNA of the initial swaps was isolated using the NucleoSpin RNA kit (Macherey & Nagel) followed by a reverse transcription utilizing the SuperScript IV together with Oligo dT and random hexamer primer (Thermo Fisher) according to the manufacturers' instructions. Subsequently, the complementary deoxyribonucleic acid (cDNA) was subjected to deep sequencing. Sequencing libraries were prepared from 4.5 µL cDNA using NEBNext ARTIC SARS-CoV-2 Library Prep Kit for Illumina sequencing platforms (catalog no. E7650; New England BioLabs Inc., neb.com). Concentration and size of the cDNA amplicons and libraries were assessed using Qubit fluorometer and Tapestation (High Sensitivity D1000 ScreenTape), respectively. High-throughput paired-end sequencing was performed using Illumina MiSeq sequencer and MiSeq Reagent Kit v2 (500-cycles) following the manufacturer's recommendations. Raw reads were quality checked, trimmed, and mapped to the SARS-CoV-2 reference sequence (NCBI Reference Sequence: NC_045512) using QIAGEN CLC Genomics Workbench 21.0.5. After removing duplicates, partially full-length consensus sequences were extracted, and samples were assigned to respective lineages using the pangolin tool [13] (Table 2). In addition, variants in spike domains were identified and annotated using Geneious prime 2021.2.2 (https://www.geneious.com).

Ethical Approval and Patient Consent

The study was conducted according to the revised principles of the Declaration of Helsinki and was approved by the ethics

committee of the Ruhr-University Bochum (registration number 20-7065) in November 2020. All patients gave written informed consent.

RESULTS

Study Cohort

A total of 15 patients (33.3% female) between 39 and 89 years (mean age 70.5 [±12.5] years) were recruited. Baseline parameters including laboratory findings on admission are presented in Table 1. All study patients had risk factors for a severe course of COVID-19 according to the criteria of the WHO [8] and RKI [14]. Three (20%) patients had up to 2 risk factors, whereas most patients had multiple risk factors for a severe COVID-19 illness (3–4 risk factors, 7 [46.7%]; 5–6 risk factors, 2 [13.3%]; >6 risk factors, 1 [6.7%]). Body temperature on admission was 37.0 (±0.9)°C. On the study day, 9 (60%) patients were categorized with a mild COVID-19 disease according to STACOB criteria (adaptation following the WHO Therapeutics and COVID-19: living guideline [8]). Nasal oxygen support was required by 7 (46.7%) patients. Mean peripheral oxygen saturation on admission was 93.4% (SEM ± 7.4%). None of the included patients were vaccinated against SARS-CoV-2.

On the day of the study, most patients had only mild symptoms (n = 9 of 15, 60%) and were categorized to mild COVID-19 disease. Follow-up revealed a clinical worsening in 10 patients and 3 died (2 patients died of COVID-19, 1 patient died with COVID-19). Consistent with literature [16], a high level of lactate dehydrogenase ($r = 0.53$, $P = 0.044$), leucocytes ($r = 0.69$, $P = 0.0056$), but also C-reactive protein ($r = 0.54$, $P = 0.035$) on admission was significantly correlated with a more severe COVID-19 infection (Supplementary Appendix Figure 2). No significant correlation was found for COVID-19 severeness and other described severeness predictors including age ($r = 0.44$, $P = 0.11$), alanine aminotransferase ($r = 0.31$, $P = 0.26$), aspartate aminotransferase ($r = 0.07$, $P = 0.81$), procalcitonin ($r = 0.53$, $P = 0.47$), or D-dimers ($r = 0.45$, $P = 0.11$).

Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load and Sequencing

The patients' viral load before sample acquisition as determined with the combined nasopharyngeal and oropharyngeal swabs are displayed in Table 2. Mean CT values in RT-qPCR analyses were E-Gene 15.3 (SEM ± 2.7), S-Gene 18.1 (SEM ± 4.9), RdRP-Gene 17.2 (SEM ± 3.7), and N-Gene 19.4 (SEM ± 5.5). Viral variants included supposed wild-type (n = 10, 66.7%), variant of concern (VoC) Alpha (n = 4, 26.7%), and VoC Beta (n = 1, 6.7%). Deep sequencing using the pangolin tool [13] confirmed lineage assignment for 14 of 15 (93.3%) samples for nearly full-length genomes. Variant patterns identified by detailed investigation of spike domains underlined lineage assignment according to RKI variant reports [17] (Figure 1, Table 2).

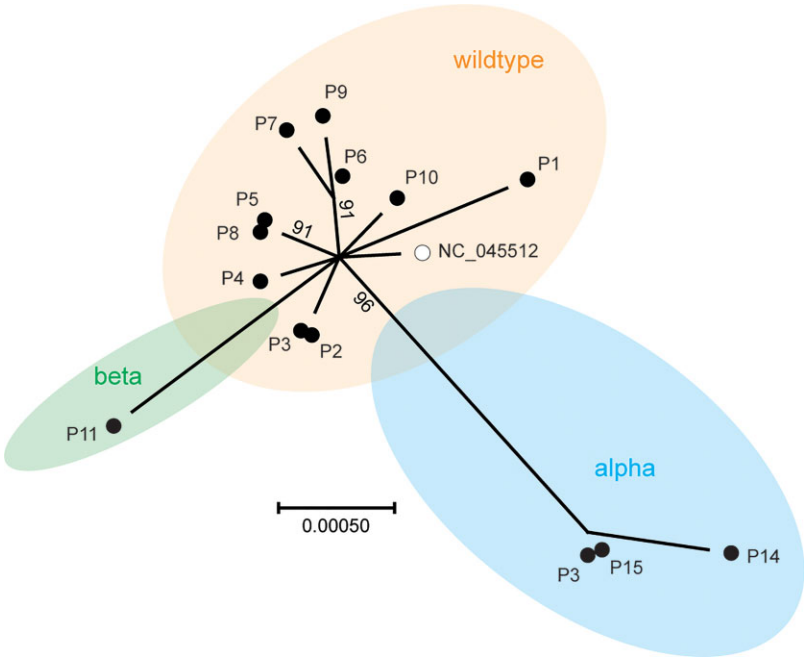

Figure 1. Viral isolates analyzed in this study. Spike domains (nt) of 14 samples (black dots) and the severe acute respiratory syndrome coronavirus 2 reference genome NC_045512 (open circle) were assembled using Clustal Omega at EMBL-EBI [18]. Phylogenetic analysis was conducted with 'One click' at Phylogeny.fr [19] and tree was visualized with MEGA X [19]. Scale bar indicates the number of changes per site in maximum likelihood inference (HKY85 substitution model); numbers at branches represent bootstrap values (1000 repetitions; cutoff ≥70%).

Two patient samples were excluded from the study due to bacterial/fungal contamination within the cultures (Supplementary Appendix Table 1). Viral RNA could be detected from all (n = 13 of 13) combined nasopharyngeal and oropharyngeal swabs (inoculum), and viral RNA could be successfully detected within the inoculated cell cultures (Figure 2, Supplementary Appendix Figure 3). After inoculation with the "swabs", viral loads in the cells ranged from 2.23×10^{1} to 2.03×10^{9} RNA copies/50 ng and in the supernatant from not detectable to 6.58×10^{7} RNA copies/mL (Supplementary Appendix Table 1). Infectious virus ("infectivity"), determined as $TCID_{50}$/mL, could be recovered from the nasal-oropharyngeal swabs from n = 6 of 13 (46.2%) patients (Figure 2; P1, P2, P3, P5, P8, P10). Of note, despite inclusion criteria defining a high viral load (CT ≤ 25; Table 2), some patients displayed lower viral loads at the time point of the study, with none of the swab samples resulting in productive virus infection in cell culture (Supplementary Appendix Figure 3; P.11, P12, P13, P14).

Evaluation of Severe Acute Respiratory Syndrome Coronavirus 2 Transmission Risk

Steel carriers contaminated via intensive moistening with saliva (Figure 2, moisten) resulted in a visible CPE and detectable viral RNA within the cells (at least 1 time point positive) in n = 6 of 13 (46.2%) cases (P1, P2, P3, P5, P9, P10). Despite the absence of a visible CPE in patient 6 and patient 10 (Figure 2, time points 30 and 45 minutes), viral RNA was detected in the cells and supernatants, and infectious virus was quantified, indicating that harvesting might have been too early for the appearance of CPE. Infectious virus was recovered from n = 5 of 13 (38.5%) contaminated steel carriers (Figure 2; "SN infectivity" P1, P3, P5, P6, P10) with viral titers ranging from 5.59×10^{1} to 8.68×10^{5} $TCID_{50}$/mL. In some patients, infectious virus could be recovered from the steel carriers for up to 240 minutes after incubation at room temperature (Figure 2; P1, P3, P5), underlining the environmental stability of SARS-CoV-2 over several hours. For other samples (Figure 2; P2, P6, P10), infectious virus could be recovered at early time points only (1 minutes, 15 minutes, 30 minutes, 45 minutes).

After contamination with coughing, viral RNA could be weakly detected within 5 of the cellular samples (Figure 2; cough P1, P7, P8, P10) (Supplementary Appendix Figure 3; P11). However, none of the contaminated surfaces, via coughing, resulted in productive cellular infection as determined by the appearance of CPE and quantification of infectivity (Figure 2, Supplementary Appendix Figure 3).

DISCUSSION

Although respiratory droplets and aerosols exhaled from infected individuals are currently considered the main route of SARS-CoV-2 transmission, the role of droplet-contaminated surfaces (fomites) as a potential source of infection remains controversial. Fomite-based transmission has been proposed to contribute to the spread of other common respiratory pathogens [20, 21], including experimental studies examining the transfer of infectious influenza viruses and/or respiratory syncytial virus between hands and surfaces [22]. However, current evidence points towards a low risk of SARS-CoV-2 transmission in this scenario [1, 23, 24], requiring a timely order of specific events [7]. To examine this potential risk of SARS-CoV-2 surface transmission, we assessed the amount of SARS-CoV-2 genomic material and infectious viral particles after contamination by individuals infected with SARS-CoV-2 over time. The results of the present study highlight that viral contamination via coughing on surfaces does not represent a major risk of transmission.

Our study cohort was characterized by nonvaccinated, hospitalized, mostly elderly patients with multiple comorbidities. Since the vaccination program in Germany started in December 2020 (during the study period), none of the patients were vaccinated against SARS-CoV-2. This cohort may therefore be quite representative for hospitalized patients during the first and second COVID-19 wave in most countries [25]. However, because the proportion of unvaccinated people in the population remains significant [26] and completely vaccinated individuals can still be infected and often present with high viral loads [27], the present results remain of significant importance for the ongoing pandemic. We were initially able to recruit patients in early stages of COVID-19 due to outbreaks in hospitals and rehabilitation facilities and subsequently early referral to the isolation ward. Later, patients were diagnosed in ambulatory settings and predominantly admitted via the emergency department, often only when clinical conditions deteriorated and ambulatory management failed. The hospitalized cohort may explain the high rate of risk factors and, consequently, follow-up mortality in the present study. However, the present cohort is characterized by high viral load. Hence, a high transmission rate can be assumed [28], supporting the main conclusion of the study. Of note, several laboratory parameters on admission significantly correlated with a severe outcome. However, the present study was not designed for this analysis, and, therefore, these correlations need to be considered exploratory.

Infectious virus could be recovered from the combined nasopharyngeal and oropharyngeal swabs and steel-carriers contaminated via intensive moistening from a significant number of patients. For some patients, infectious virus could be recovered for up to 240 minutes (Figure 2). This demonstrates that infectious virus can be transferred from saliva by moistening onto surfaces from patients and can be recovered for several hours. As described previously, we did not observe differences of the viral stability between the wild-type and VoCs (Alpha

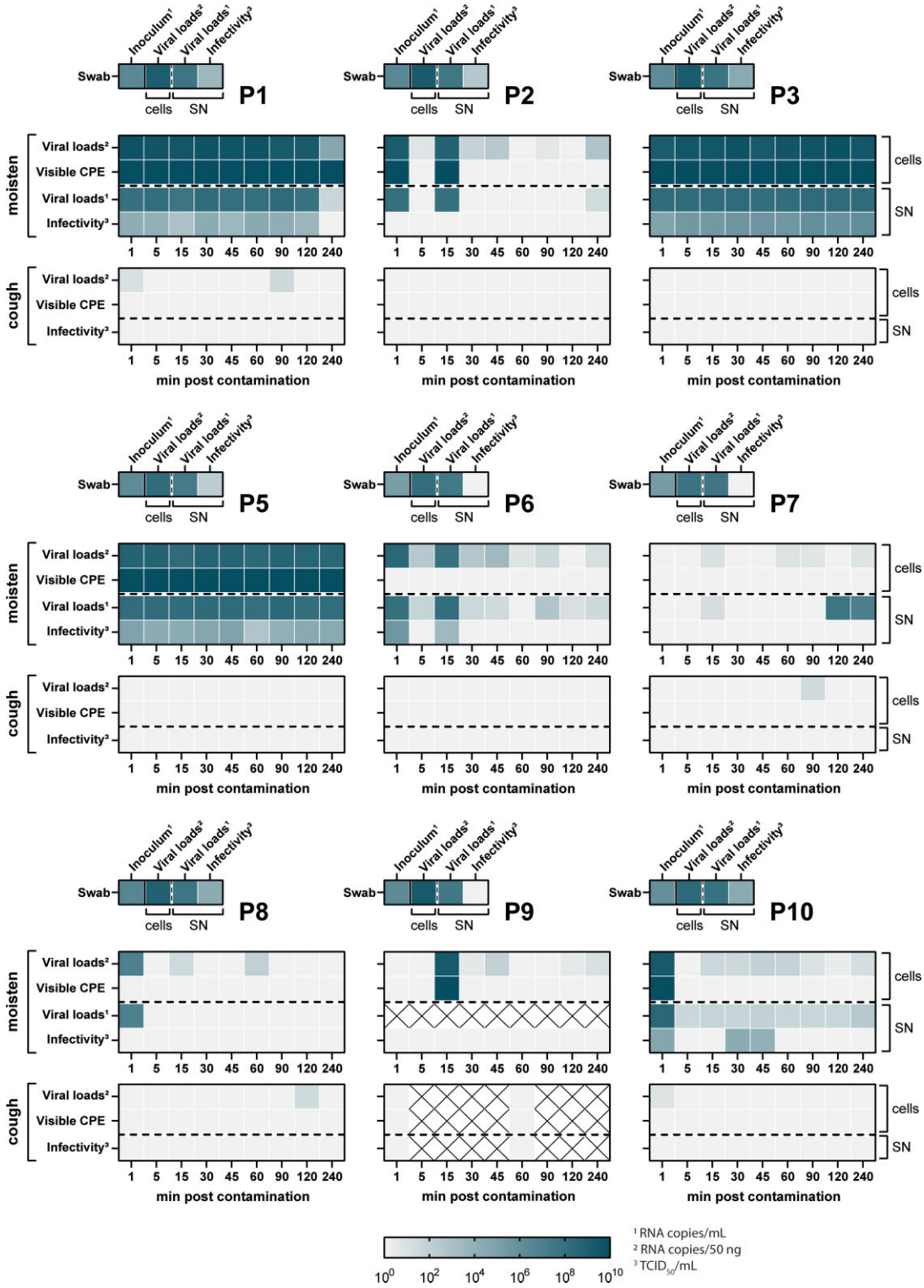

Figure 2. Quantification of viral loads and infectivity of patient swabs, moisten samples, and cough samples that could be successfully recovered in cell culture. VeroE6 cells were inoculated with the patient material and monitored on a daily basis. Upon the emergence of cytopathic effects, the supernatant was collected to determine viral loads by quantitative reverse-transcription polymerase chain reaction (RT-qPCR) (ribonucleic acid [RNA] copies/mL; indicated by[1]) and viral titers by an endpoint-dilution assay ($TCID_{50}$/mL, indicated by[3]). In addition, RNA was isolated from the cells and subjected to RT-qPCR to determine viral loads (RNA copies/50 ng total RNA, indicated by[2]). For each patient (P1–P10), 3 panels were designed. The top small panel exclusively includes data regarding the patient swabs, whereas the larger middle panel shows the data for the moisten samples, and the lower panel shows the data collected from the cough samples. For moisten and cough samples, viral loads and infectivity at 9 different time points were determined. The color indicates the amount of virus being detectable in each sample, with light gray being the lower limit of detection to dark blue resulting in 10^{10} RNA copies/mL, RNA copies/50 ng, or $TCID_{50}$/mL. The visible cytopathic effect (CPE) was rated 2 dimensionally, with light gray being "no visible CPE" and dark green being "visible CPE".

and Beta), implying a comparable environmental stability [29]. The stability of SARS-CoV-2 on surfaces is likely determined by a combination of factors, including the initial amount of infectious virus deposited, possible presence of antibodies within the sputum and environmental parameters. Given the controlled laboratory conditions for virus recovery as herein presented (eg, large inoculums, small surface area, no ultraviolet exposure), the viral survival observed might therefore differ from real-life scenarios, necessitating careful interpretation. For example, a recent study observed a low transfer efficiency between different surfaces and fingertips after an initial drying of an inoculum with a low viral titer (1×10^4 $TCID_{50}$/mL) [24]. Hence, even if sufficient viable virus is deposited on a surface, a timely contact and high transfer efficiency are required to transfer an infectious dose, which subsequently needs to be exposed towards susceptible tissues (eg, mucosa, eyes). More importantly, we did not observe the recovery of infectious virus after patients coughed onto a surface, implying that droplet-contamination of surfaces does not present a major transmission route for SARS-CoV-2. Given that nonhospitalized and presymptomatic, asymptomatic, and mildly symptomatic individuals across different age groups frequently display viral loads within a comparable range [30], similar observations as herein observed for elderly and hospitalized patients can be inferred. Of note, a similar study was undertaken during the original SARS epidemic of 2002–2004, caused by SARS-CoV-1. Dowell et al [31] took smears from hospitalized SARS patients, placed those smears on surfaces, then assayed for viral RNA and infectious virus. Although they did find viral RNA, no infectious virus was recovered from the surfaces, which is in line with the results obtained in this study.

Our study encompasses several limitations. Patients were encouraged to forcefully cough twice to contaminate surfaces. However, we cannot exclude that potentially repeated coughing over a prolonged time results in a more effective virus transfer compared with our controlled conditions. Moreover, sneezing can produce significantly more infectious droplets potentially containing infectious particles; therefore, we cannot exclude potential transmissions via this route. Furthermore, a selection bias cannot be excluded, and the included patients are not demographically representative. Strengths of the present study include the high viral load of the patients included, a standardized protocol for sample acquisition, laboratory procedures, and the inclusion of VoC.

CONCLUSIONS

The present study provides evidence that fomites may not be as critical in the transmission of SARS-CoV-2 as initially suspected. However, the present study also provides evidence that infectious SARS-CoV-2 can be found on some fomites for a relatively long period of time after contamination with extensive amounts of saliva. Therefore, common hygiene practices (eg, coughing/sneezing into elbows, hand hygiene) should still be considered to avoid surface contamination and virus transfer. Face masks may further mitigate the risk of fomite transmission. Taken together, our findings suggest that fomites contaminated with coughing are unlikely to be an important source of SARS-CoV-2 transmission.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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