Investigating the presence of SARS-CoV-2 on the Surfaces, Fomites, and in Indoor Air of a referral COVID-19 Hospital in a Middle Eastern Area

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Abstract

Coronavirus illness (COVID-19) is an immensely transmissible viral infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This study aimed to assess the presence of SARS-CoV-2 in the indoor air, on the surfaces, and the fomites, and in the indoor air of a COVID-19 referral hospital in Shiraz, Iran. Indoor air sampling was conducted utilizing a standard midget impinger contained 15 ml of viral transfer medium (VTM) equipped with a sampling pump with a flow rate of 10 L min^{-1} for 60 minutes. Surfaces and fomites were sampled using sterile polyester swabs. The RNA of SARS-CoV-2 was detected in about 41.2% of indoor air and 32% of swab samples. Four out of the six (66.7%) indoor air samples up to a distance of 2 meters from the patient's bed in intensive care units (ICU-1, ICU-3), accident and emergency (A&E-2), and negative pressure room were positive for SARS-CoV-2 RNA. All air samples within 2 to 5 meters from the patient's bed were negative. This study's results did not support the airborne SARS-CoV-2 transmission; However, it showed contamination of surfaces and fomites in the studied hospital's wards.

1. Introduction

COVID-19 is an immensely contagious respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first officially reported in late 2019 in Wuhan, Hubei Province, China (Andersen et al. 2020, Hu et al. 2020, Read 2020). It quickly spread to numerous countries worldwide and has been declared a global pandemic by the World Health Organization (WHO) (Shakoor et al. 2020). The first COVID-19 case in Iran was officially reported on February 19, 2020; by the ministry of health and medical education (MOHME). In the first ten days of the disease epidemic, the reproductive number (R_0) for SARS-CoV-2 was 4.70 (95% CI: 4.23–5.23) (Azimi et al. 2020).

At the beginning of the SARS-CoV-2 epidemic, the knowledge on transmission patterns was based on the existing literature on viruses from the same family, such as the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1). Following the 2015 outbreak of MERS-CoV in the Republic of Korea, several studies were carried out to assess MERS-CoV's survival and stability in the air and on the surfaces in healthcare facilities. In a study carried out by bin et al., the RNA of MERS-CoV was isolated up to five days from surfaces after the last positive polymerase chain reaction (PCR) test from respiratory samples of patients. Moreover, viral RNA has been isolated in several surface samples from anterooms, medical tools, ventilation systems, bedsheets, bedrails, intravascular fluid hangers, and X-ray imaging equipment. Kim et al. observed the uninjured MERS-CoV particles of viral cultures of the indoor air and surface samples using the electron microscope imaging technique (Bin et al. 2015, Kim et al. 2016).

When someone with respiratory tract infections (RTIs) breathes, talks, shouts, sneezes, or coughs, the infectious large and small droplets are released into the environment. These virus-containing respiratory droplets of various sizes can be suspended through the atmosphere for minutes to hours due to complex movements and thermodynamic transformations. They can be emitted in the environment depending on airflow, temperature, and relative humidity (Moreno et al. 2021). There are three main modes that respiratory viruses are transmitted. First, person-to-person transmission occurs via direct contact with an infected individual or indirectly by touching contaminated surfaces/fomites. Second, transmission through large or small virus-containing respiratory droplets, which occurs in the vicinity of the infected individual. Third, airborne transmission of suspended droplets or particles (The Lancet Respiratory Medicine 2020). Studies on the transmission routes of SARS-CoV-2 have indicated that it could be transmitted from person to person via expelled respiratory droplets larger than 5 μm in diameter. Besides, close contact (within 1 m) with an infected COVID-19 patient could be another transmission route for SARS-CoV-2. The viral transmission also occurs indirectly due to contact with contaminated surfaces/fomites (Setti et al. 2020a, World Health Organization 2020c).

The airborne transmission terminology in the case of viruses, especially the novel SARS-CoV-2, refers to the infection caused by exposure to tiny respiratory droplets or fine particles containing the virus, which can remain suspended in the air for extended periods and prolonged distances, usually more than two meters (Scientific Brief Updated Oct. 5, 2020). Several studies have explained the airborne transmission route of Norwalk-like virus and SARS-CoV-1 in school children, hospitals, health care facilities, and airplanes (Booth et al. 2005, Li et al. 2005, Morawska &Cao 2020, Olsen et al. 2003). The result of a study conducted by Hongna Zhang et al. in pig farm revealed that the H1N1 virus has the ability to be contagious to animals via airborne routes (Zhang et al. 2013).

By implementing social distance, quarantine, and home isolation programs according to the WHO and CDC guidelines, and because of the numerous similarities between the two SARS viruses, there is a hypothesis that SARS-CoV-2 airborne transmission is a possible way to spread COVID-19 when masking or covering of the face is not applied (Fineberg &Council 2020, Zhang et al. 2020).

Current investigations have isolated SARS-CoV-2 RNA from aerosols in Wuhan hospitals and outdoor air in Italy (Liu et al. 2020b, Setti et al. 2020b). Moreover, Viable SARS-CoV-2 was detached from air samples at a distance of 2–4.8 m from patients (Lednicky et al. 2020). Nonetheless, SARS-Co-V-2 was not detected in the air of patient's rooms in a study by Sean Wei Xiang Ong et al. Similar finding was reported by Y. H. Li et al (Li et al. 2020). Besides, in a study conducted by Faridi et al. in the indoor air of patients' rooms, all collected indoor air samples at the distance of 2 to 5 m from the patients' beds were negative for SARS-CoV-2 RNA (Faridi et al. 2020, Ong et al. 2020). To date, the airborne transmission is not understood, and there is controversy regarding the airborne transmission of COVID-19 (Tabatabaeizadeh 2021). However, the Centers for Disease Control and Prevention (CDC) and the WHO guidelines suggested that the chance of SARS-CoV-2 airborne transmission can happen under special circumstances and with aerosol-generating procedures, but there is controversy regarding the airborne transmission route of SARS-CoV-2. According to the CDC, special conditions are indoor with improper ventilation and exposure to an infected individual lasting more than 30 minutes (Ram et al. 2021, The Lancet Respiratory Medicine 2020, Wilson et al. 2020).

In previous SARS and MERS epidemics, healthcare workers (HCWs) accounted for 21% of cases. Moreover, in Hong Kong, Singapore, and Canada, over half of all mortality due to SARS infection occurs among HCWs (Bahl et al. 2020). Hence, HCWs have the highest risk of exposure to SARS-CoV-2 (Adams &Walls 2020). In China, approximately 3,000 HCWs infected with SARS-CoV-2, and 22 were died due to COVID-19 (Sim 2020). It has been estimated that 10 to 20 percent of all COVID-19 infection cases occur among the front-line HCWs, which means maintaining their safety is critical (Nguyen et al. 2020). Intensive care units (ICUs), because of the high presence of viruses in the environment and infected medical equipment, are the most common infection source in healthcare settings. Therefore, the HCWs who treat patients in ICUs are at risk of COVID-19 infection (Pourdowlat et al. 2020). Hence, investigating transmission patterns of the virus in healthcare facilities would be crucial to protecting the HCWs from exposing to SARS- CoV-2. The present study aimed to investigate the possible airborne transmission of SARS- CoV-2 and the virus's presence on the surfaces and fomites in a referral COVID-19 hospital in the Middle East.

2. Materials And Methods

2.1 Area of study

This cross-sectional study was conducted in July 2020 at the Ali Asghar Hospital in Shiraz county, Iran, considered the referral hospital for hospitalization of COVID-19 patients by the Shiraz University of Medical Sciences.

Shiraz, the capital of Fars province, is placed at the latitude of 29°36' N and the longitude of 52°32' E (northwest of Fars Province, south of Iran) with an average height of 1500 m above sea level and area of 340 km^2 (Fig. 1). Shiraz is the fifth-most-populous city in Iran with almost two million populations (Gharehchahi et al. 2013, Shahsavani et al. 2017).

Ali Asghar Hospital has several wards including, triage, two accident and emergency (A&E) wards, five inpatients' wards, three ICU, one negative pressure room, computed tomography scan (CT scan), laboratory, and an administrative department.

2.2 Environmental sampling

2.2.1 Indoor

In this study, 17 indoor air samples were collected using a standard midget impinger and a sampling pump (Legend Legacy, SKC Inc.) with a flow rate of 10 L min^{-1}. The indoor air sampling was performed for 60 minutes. Previous studies reported the impinger technique as a successful method to collect the SARS-CoV-1 from the air (Dart &Thornburg 2008, Verreault et al. 2008). Before each sampling campaign, the impinger and the connecting tube were disinfected using ethyl alcohol 70%. Besides, the impinger was contained 15 ml of viral transfer medium (VTM). The VTM was contained Dulbecco's Modified Eagle Medium (DMEM), streptomycin (100 µg/ml), penicillin (100 IU/ml), and isoamyl alcohol (1%); as an anti-foaming agent (Faridi et al. 2020).

Figure 2 shows the schematic of air sampling locations in multiple wards of the studied hospital. Air sampling equipment was set up in different wards at an altitude of 1.5 m above the ground level as follows:

- At a distance of 2 m from the patient's bed in the ICU-1, ICU-2, ICU-3, the negative pressure room, the A&E-1, and A&E-2 wards.
- On the nursing station's counter (2 to 5 m from the patient's bed).
- In the admission and discharge office, inpatient's room, CT scan, laundry, office room in the administrative department, and temporary waste storage area (TWS).

2.2.2 Surface and fomites

Twenty-two samples were collected from surfaces and fomites using sterile polyester tipped swabs (Dacron). Each collection vial contained 3 ml of the VTM. The surfaces were sampled before the daily cleaning and disinfection program. According to the WHO recommended protocol, the surface sampling procedure was performed with an area of 25 cm^2 for swab sampling (World Health Organization 2020a).

2.3 SARS-CoV-2 virus detection test

The real-time reverse transcription-polymerase chain reaction (rRT-PCR) is currently the gold standard and most reliable SARS-CoV-2 detection method, which relies on recognizing unique ribonucleic acid (RNA) sequences in the SARS-CoV-2 (COVID LabCorp, World Health Organization 2020b).

All the SARS-CoV-2 samples were immediately shipped to laboratory under cold conditions (4°C) for further analysis. Collected samples were initially placed in a 50 ml Falcon containing phosphate-buffered saline (PBS) solution and stored in a refrigerator at 4°C. The SRAS-CoV-2 virus was then isolated using a virus RNA extraction kit (PCR cloning kit, SINACLON Co.-Iran) based on the manufacturer's instruction. Figure 3 shows the PCR product cloning procedure. All samples were centrifuged at 13000 RPM. Finally, the isolated RNA was tested using one-step rRT-PCR for the SRAS-CoV-2 virus.

According to the manufacturer's instructions, the presence of the SARS-Cov-2 genome was assessed using a commercially available SARS-CoV-2 Test Kit (Pishtaz Teb Zaman Co.-Iran), according to the manufacturer's instructions using one-step plus Real-Time PCR method (Applied Biosystems, USA). The SARS-CoV-2 Test Kit was a molecular in vitro diagnostic technique that uses TaqMan probe-based technology for the qualitative detection of SARS-CoV-2. The nucleocapsid (N) genes and RNA-dependent RNA polymerase (RdRp) were the virus detection targets. In addition, Ribonuclease P (RNase P) was used as an internal control.

3. Results And Discussion

3.1 Results of indoor air sampling

The results of presence or absence of SARS-CoV-2 RNA in indoor air in different wards at the studied hospital are shown in Table 1. The RNA of SARS-CoV-2 was found in 41.2% of indoor air samples. Typically, after screening and triage, patients with COVID-19 symptoms are admitted to healthcare settings, implying the staff at the admission and discharge office be in close contact with COVID-19 patients. In this study, one indoor air sample collected from the admission and discharge office showed the presence of the SARS-CoV-2 RNA. Additionally, indoor air samples collected at a distance of 2 m from the patients' beds in ICU-1, ICU-3, and A&E-2 were positive. All collected indoor air samples from the nursing station over the distance of 2 to 5 m from the patient's bed were negative. The air sample taken from a crowded office in the administrative department was positive, which could be due to the occasional presence of HCWs, and inadequate ventilation (Somsen et al. 2020). Besides, indoor air samples were positive for TWS. The RNA of the SRAS-CoV-2 at a distance of 2 m from the patients' beds has been detected in the indoor air of the negative pressure room, probably due to the inadequate air exchanging provided by the ventilation system (Jayaweera et al. 2020). WHO recommended the minimum hourly averaged ventilation rates of 160 l/s/patient, 60 l/s/patient, and 2.5 $l/s/m^3$ for airborne precaution rooms, general wards and outpatient departments, and corridors, respectively (Chartier &Pessoa-Silva 2009).

Table 1
Results of indoor air sampling in different wards of the hospital.

Samples	Hospital departments	No. of patients or staff/status	Presence or absence of SARS-CoV-2	Ventilation system	Temperature (°C)	Relative humidity (%)
1	ICU-1	10 (Oxygen mask: 6, Intubated: 4)	+	Mechanical	23.4	30.6
2	ICU-2	10 (Oxygen mask: 5, Intubated: 5)	-	Mechanical	23.0	31.0
3	ICU-3	7 (Oxygen mask: 5, Intubated: 2)	+	Mechanical	24	31.0
4	Negative pressure room	2 / Intubated	+	Mechanical	23.1	19.0
5	A&E-1	15 (Oxygen mask: 2, Masked:13)	-	Mechanical/Natural	22.8	33.0
6	A&E-2	15 (Oxygen mask: 1, Masked: 14)	+	Mechanical/Natural	25.4	30.8
7	A&E-1 (Nursing station)	4 / Masked	-	Mechanical	21.8	27.6
8	A&E-2 (Nursing station)	4 / Masked	-	Mechanical	23.0	27.5
9	Surgery ward (Nursing station)	3 / Masked	-	Mechanical	24.4	32.0
10	Men’s Internal Ward	4 / Masked	-	Mechanical/Natural	21.0	27.6

Samples	Hospital departments	No. of patients or staff/status	Presence or absence of SARS-CoV-2	Ventilation system	Temperature (°C)	Relative humidity (%)
11	Men’s Internal Ward (Nursing station)	3 / masked	-	Mechanical/Natural	22.5	29.0
12	Woman’s Internal Ward	4 / masked	-	Mechanical/Natural	23.0	33.7
13	CT Scan	1 / masked	-	Mechanical	25.0	19.8
14	Admission and discharge office	2 / masked	+	Natural	19.0	21.0
15	Office in the administrative department	3 / masked	+	Natural	23.2	26.2
16	Landry	2 / masked	-	Mechanical	29.0	50.6
17	TWS	1 / masked	+	Mechanical	27.6	41.7

Previous studies suggested that airborne transmission could be considered an important transmission mode for the SARS-CoV-2 in healthcare settings (Liu et al. 2020a). Similar findings were reported in a study conducted at the Nebraska University Hospital in the United States (Santarpia et al. 2020). In a study performed in a hospital in Milan, Italy, all indoor air samples collected from the ICU and the corridor were positive for SARS-CoV-2 (Razzini et al. 2020). An experimental study has reported the number of droplets of different sizes remaining in the air during regular communication (Asadi et al. 2020, Stadnytskyi et al. 2020). Another experimental model showed that healthy people could produce aerosols by coughing and speech (Somsen et al. 2020). The airborne transmission of SARS-CoV-2 can be influenced by different parameters such as relative humidity and temperature. At a relative humidity below 40%, the probability of airborne transmission of SARS-CoV-2 is higher than the humidity of 90% or greater. The persistence of the SARS-CoV-2 at low temperatures (4°C) is significant, and at temperatures around 70°C, it can no longer be detected after 5 minutes (Ahlawat et al. 2020, Delikhoon et al. 2021). The ability of aerosols and small droplets to disperse increases in the atmosphere when the size declines, such that the transmission distance of the aerosols may exceed 2 meters from an infected person (Godri Pollitt et al. 2020). Numerous similarities between the two SARS-CoV-1 and SARS-CoV-2 and the available information on viruses' transmission, in general, support the hypothesis that COVID-19 is emitted through the air over 2 meters away from the infected person (Morawska &Cao 2020, Nissen et al. 2020, Setti et al. 2020a). Nonetheless, in a study conducted by Faridi et al. (2020) in the indoor air of patients' rooms with severe and critical symptoms of COVID-19, all collected indoor air samples at the distance of 2 to 5 m from the patient's bed were negative in terms of SARS-CoV-2 (Faridi et al. 2020), which is consistent with the results of our study. Besides, SARS-Co-V-2 was not detected in the air of patients' rooms in a study by Sean Wei Xiang Ong (Ong et al. 2020).3.2 Results of surfaces and fomites sampling

Many studies on the airborne transmission of the SARS-CoV-2 have used the RT-PCR test for diagnosis. Nevertheless, the RT-PCR test detects only SARS-CoV-2 RNA, not the viable or infectious form of virus (Sepulcri et al. 2021). Therefore, this technique cannot express the infectious ability of the virus (Ram et al. 2021).

Table 2 shows the surfaces and fomites sampling results. The results showed that 32% of the surface and fomite samples were positive. Also, 33% of samples taken from the medical ventilator and the patient’s bed surface in the ICU wards were positive. Besides, all samples collected from the exhaust fans in ICUs and A&Es were negative. While in a study carried out by Karolina Nissen et al. in the wards at Uppsala University Hospital, Sweden, 88.9% of Samples from the main exhaust filters were positive. Hence, they suggested that there could be a potential for airborne transmission of SARS-CoV-2 (Nissen et al. 2020). Surfaces that were more frequently touched, such as doorknobs in the bathroom of ward-1were also positive. Samples from the food trolley were positive, suggesting that the virus infection may be transmitted from the wards to the hospital’s kitchen. The surface sample collected from the protective counter screen in the admission and discharge office was positive for the SARS-CoV-2. Therefore, special attention should be paid to the disinfection of surfaces in the hospital admission and discharge office.

Table 2
Results of presence of SARS-CoV-2 RNA in surface and fomites samples of different hospital wards.

samples	surfaces and formats	presence or absence of SARS-CoV-2 virus
1	Medical ventilator (ICU-1)	+
2	Patient’s bed (ICU-1)	+
3	Medical ventilator (ICU-2, ICU-3)	-
4	Patient’s bed (ICU-2, ICU-3)	-
5	Exhaust fan (ICU-1, ICU-2, ICU-3)	-
6	The surface of the counter of the nursing station (Triage)	+
7	Patient’s bed (Ward-1)	-
8	Office desk (Ward-1)	-
9	Exhaust fan (Ward-1 and Ward-2)	-
10	Doorknobs (Bathroom/Ward-1)	+
11	Hospital bedsheet (Landry)	-
12	CT scan patient’s bed	-
13	Admission and discharge office (Protective Counter Screen)	+
14	Hospital food trolley surfaces	+
15	TWS area surfaces	-
16	Medical waste autoclaves	-

Razzini, K. et al. (2020) showed that 24.3% of the swab samples collected from polluted areas in the ward of a hospital in Milan, Italy, were positive for SARS-CoV-2 RNA (Razzini et al. 2020). Likewise, 34.1% of samples collected from frequently-touch surfaces during patient incubation were positive for SARS-CoV-2 RNA in a study performed by Xiaowen Hu. et al. (2020) in Qingdao, China. In that study, the collected samples from bathrooms and bedrooms were positive, with the rate of 46.7% and 50.0%, respectively. Furthermore, 60%, 40%, 40%, 33.3%, and 16.7% of the samples were positive for cotton, ceramics, metal, wood, and plastics, respectively. All surface samples in the living room were negative (Hu et al. 2021). In another study at a hospital in China, on-site SARS-CoV-2 contamination analysis was performed by the extraction-free loop-mediate isothermal amplification (LAMP) detection method. The results showed that the surface contamination rate was more than 70% in the isolation wards (Wan et al. 2021). Besides, the percentage of contaminated surfaces and fomites with the SARS-CoV-2 is consistent with other studies' results other studies.

In the indirect transmission of the COVID-19, the material types of surface and fomites and the persistence of SARS-CoV-2 play an important role (Aydogdu et al. 2021). Several studies have reported temperature and humidity as the key factors affecting coronavirus survival on surfaces. Prior experience with SARS-CoV-1 indicated that the infection disappeared as the temperature increased in Summer (Iqbal et al. 2020, Shahzad et al. 2020a, Shahzad et al. 2020b). In addition, parameters such as temperature and humidity along with air quality may boost or diminish the transmission rate and viability of the viruses in the atmosphere or on surfaces (Doğan et al. 2020, Fareed et al. 2020). Studies in China reported a positive association between temperature and the spread of SARS-CoV-2 in Hubei, Hunan, and Anhui, provinces while negative association was found in Zhejiang and Shandong provinces (Shahzad et al. 2020a). On the other hand, some studies performed in China and the United States did not find significant correlations between ambient temperature and cumulative incidence of COVID-19 (Doğan et al. 2020, Iqbal et al. 2020, Yao et al. 2020).

In the case of SARS-CoV-2 detection, generally, the RT-PCR technique is applied for recognizing the presence of the virus, but this method only detects RNA of virus and cannot detect the viable forms. Therefore, the viability of SARS-CoV-2 on surfaces is not well understood. Chin et al. have demonstrated that on surgical masks and cloth at 22 °C, the SARS-CoV-2 survive for 96 h and 24 h, respectively (Chin et al. 2020). On the other hand, Van Doremalen et al. compared the viability of SARS-CoV-1 and SARS-CoV-2 on surfaces and aerosols under experimental conditions, and findings have the same stability of both viruses in the environment (Van Doremalen et al. 2020). The findings of a study performed by Harvey et al. revealed that the risk of COVID-19 infection from contact with high touch surfaces was less than 5 in 10,000, and quantitative microbial risk assessment has shown the negligible role of fomites in public transmission via SARS CoV-2 (Harvey et al. 2021). According to the WHO Scientific Brief update of 9 July, 2020, no evidence directly demonstrated transmission of COVID-19 from fomites. Nevertheless, the WHO considers surface contamination as the main transmission route (World Health Organization 2020d).

4. Conclusion

The present study aimed to study the potential presence of SARS-CoV-2 in the indoor air, on the surfaces, and fomites of a referral COVID-19 hospital in Iran.

Our findings showed that 33% of the indoor air samples collected within 2 m of the patient's bed were positive, indicating the risk of SARS-CoV-2 transmission from a COVID-19 patient to a healthy person at a distance of 2 m or less. Moreover, findings demonstrated that all collected indoor air samples over the length of 2 to 5 m from the patient's bed were negative. Given that the office's air sample was positive, the free movement of HCWs who are in close contact with the patients must restrict to this department, and natural or mechanical ventilation should be provided. Our results showed the contamination of surfaces and fomites in the studied hospital's wards. In the COVID-19 pandemic, there is still initial uncertainty regarding transmission routes. Hence, regular disinfection should be performed with particular attention to frequently touched surfaces in healthcare facilities. Personal protective equipment is highly recommended for all HCWs in healthcare settings and always should follow the WHO's updated guidelines. Also, air recirculation should be avoided and proper natural and artificial ventilation should be provided to reduce COVID-19 transmission in healthcare settings.

According to the WHO recommendations, keeping physical distance, wearing a mask, frequent handwashing, and providing adequate ventilation in enclosed spaces as the possible ways to help prevent the spread of COVID-19 in the community and protecting the general public should be taken seriously.

Further research should be conducted to address the possible presence of viable SRAS-CoV-2 on small droplets and aerosols in healthcare facilities and public environments.

The limitation of this study was that the RT-PCR test only detects SARS-CoV-2 RNA and cannot detect the viable virus, and also, in the RT-PCR test, false-negative results occur in a low viral load.

Declarations

Ethical approval

This research was originally approved by the Shiraz University of Medical Sciences (SUMS) with the code IR. SUMS.1399.430.

Consent to participate

Not applicable

Consent to publish

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interests

The authors declare they have no potential or actual conflicting financial or personal interests.

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Authors’ contributions

Mohammad Hoseini: Conceptualization, Methodology, Investigation, Supervision Ehsan Gharehchahi: Data curation, Writing-Original draft preparation, Sampling Fatemeh Dehghani: Prepare the viral transfer medium, Sampling Marzieh Jamalidoust: Prepare the viral transfer medium, Experiments Ata Rafiee: Interpretation of the results, Validation, Final Reviewing and Editing. All authors have read and approved the final manuscript.

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Figures

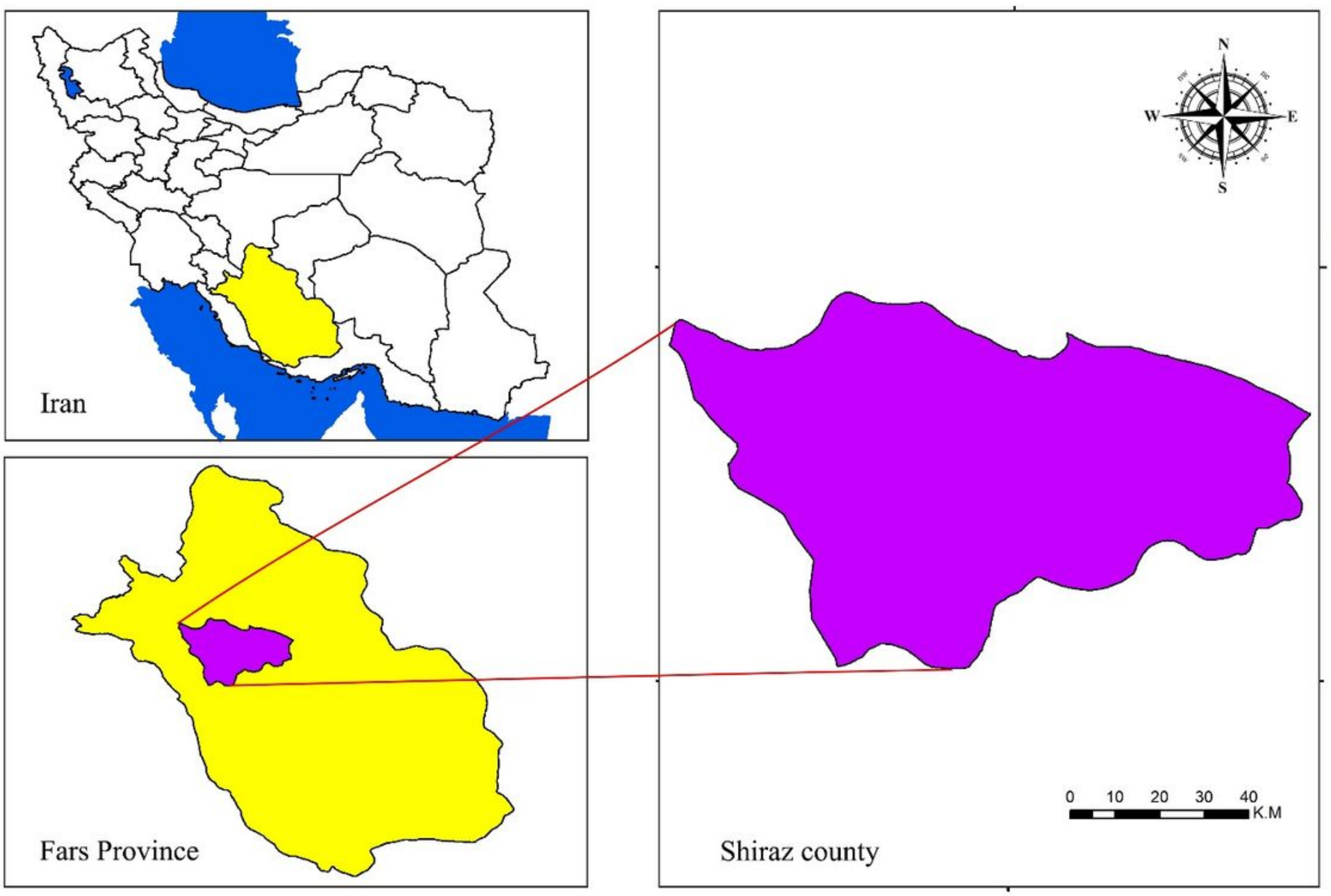

Figure 1

Location of the province of Fars and the county of Shiraz. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

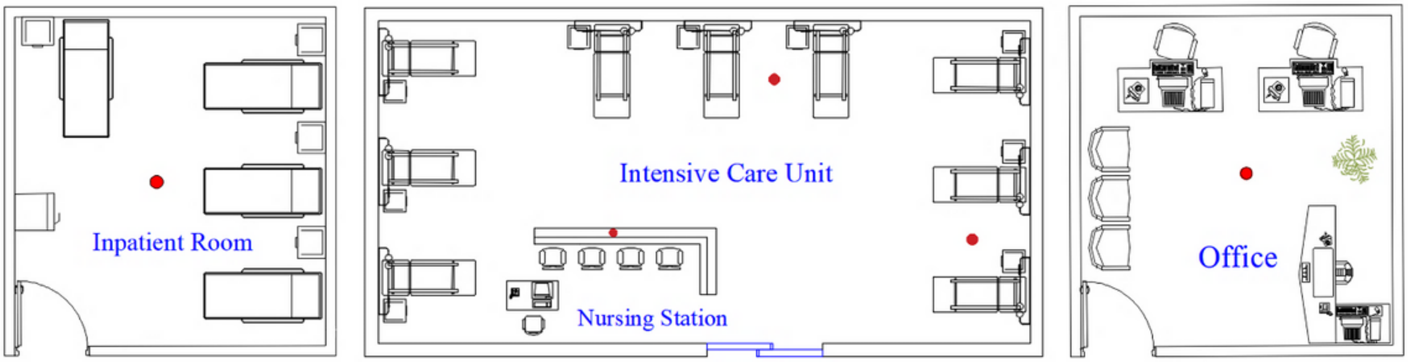

Figure 2

Schematic of air sampling locations in different wards of the hospital (red points are sampling locations).

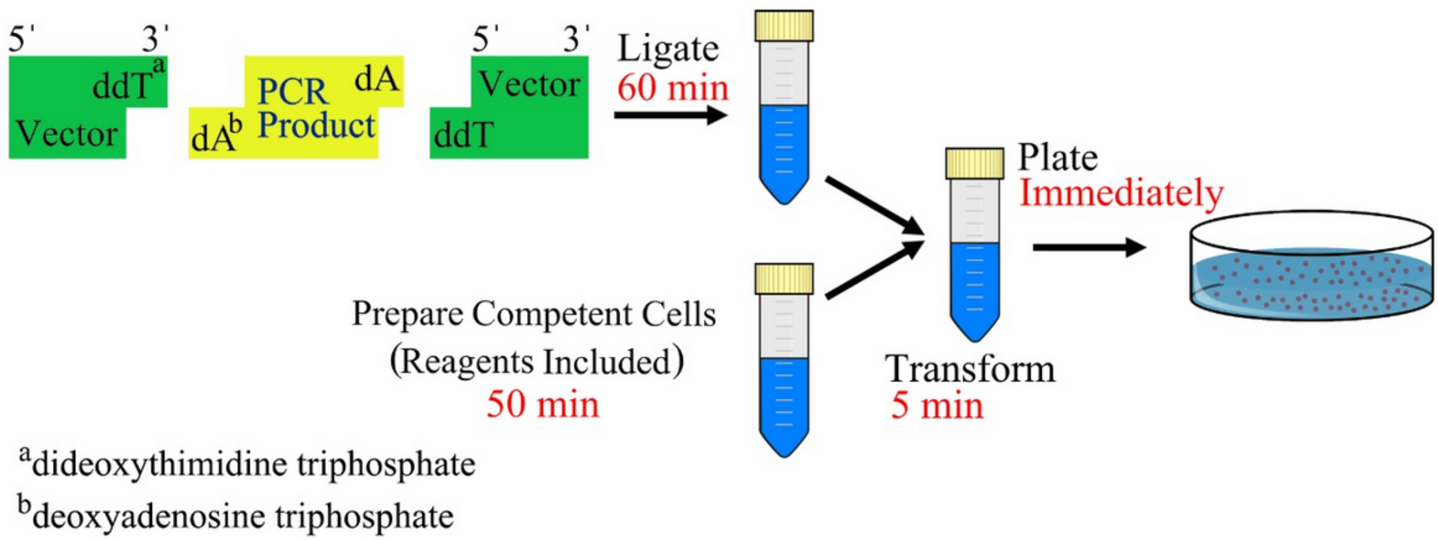

Figure 3

The PCR product cloning procedure (manufacturer's instruction).