



Research Article

Microbial Detection from Used Face Masks and Hygiene Practices

Zobiakhlui Chhakchhuak¹, Mala Chhabra², Sanjeet Panesar³, Nandini Duggal⁴

¹Department of Microbiology, ²Department of Microbiology, ³Department of Community Medicine, ⁴Department of Microbiology, ABVIMS and Dr Ram Manohar Lohia Hospital, New Delhi, India.

DOI: <https://doi.org/10.24321/0019.5138.202363>

I N F O

Corresponding Author:

Mala Chhabra, Department of Microbiology, ABVIMS and Dr Ram Manohar Lohia Hospital, New Delhi, India.

E-mail Id:

malachhabra@yahoo.co.in

Orcid Id:

<https://orcid.org/0000-0003-2328-9583>

How to cite this article:

Chhakchhuak Z, Chhabra M, Panesar S, Duggal N. Microbial Detection from Used Face Masks and Hygiene Practices. J Commun Dis. 2023;55(4):111-118.

Date of Submission: 2023-09-16

Date of Acceptance: 2023-11-25

A B S T R A C T

Background: The use of face masks for preventing the transmission of respiratory illnesses has proven to be quite effective in view of recent events. However, guidelines on proper use of face masks and hygiene practices tend to be overlooked raising the concern of potential secondary infections. Hence, the purpose of this study was to investigate the number of microbial isolates and hygiene practices in regard to mask usage.

Methods: An observational study was conducted in the Microbiology Department at ABVIMS & Dr RML Hospital, New Delhi in which 47 used face masks were collected from individuals with varying occupations for 4 months. A pre-designed, pre-tested questionnaire was used. Bacterial and fungal culturing were done as per microbiological standards. Molecular PCR was performed for SARS-CoV-2 virus detection. Chi-square test was used to test associations between qualitative variables.

Results: Cloth masks (23, 48.94%), surgical masks (13, 27.66%) and N95 masks (11, 23.40%) were collected. On observation, proper wearing of masks was higher among healthcare workers as compared to other study groups of drivers, hawkers and maids (p value: 0.025). The proportion of masks showing culture positivity showed a drastic rise in relation to the duration of usage of masks. Ninety-three bacteria were isolated with coagulase-negative *Staphylococcus* (CoNS) (37, 39.78%) constituting the majority. *Aspergillus* species (18, 48.64%) was the predominant fungal isolate.

Conclusion: According to this study, microbial isolates increase with an increased duration of wear. Good practices regarding the use of face masks and hygiene need to be promoted.

Keywords: Face Mask, Hygiene, Microbial, Pandemic



Introduction

March 2020 marked the pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the race to control the viral transmission was in full effect. As there was no definitive treatment, the greatest emphasis was placed on the prevention of transmission of the virus. One of the most effective and easiest methods for controlling the spread was by appropriate use of face masks. World Health Organization (2020) and the Ministry of Health and Family Welfare, Government of India issued guidelines on the use of medical masks on a risk-based approach.^{1,2} In the community, non-medical masks (cotton masks) serve as an alternative to medical masks. However, it was also stated that using masks alone is insufficient and has to be incorporated with hygiene etiquettes like hand hygiene, respiratory etiquette, social distancing, and appropriate use, storage, washing and disposal of masks.¹ Nonetheless, wearing masks for a long duration also raised the concern of infections and potential side effects arising from contaminated masks.³ The microbiome of the face is composed of various organisms like coagulase-negative *Staphylococcus* (*Staphylococcus epidermidis* and *S. hominis*), *Micrococcus* spp., coryneform bacteria (*Corynebacterium* spp., *Propionibacterium* spp. and *Dermabacter* spp.) and occasional *Acinetobacter* spp., which on extended use of masks raise the question of possible secondary infections with individuals' own flora.^{4,5}

In the initial phase of the pandemic, due to its rapid spread, increased transmission, and the fear of acquiring the disease, there was an increased use of N95 masks resulting in its shortage. Subsequently, the guidelines recommended judicious use of PPE and N95 masks for healthcare workers, and surgical and cloth masks for use in the general community. However, the use of cloth masks may provide a thriving environment for microorganisms to grow due to their ability to retain moisture and hence, it is important to practice good hygiene to prevent skin, respiratory and digestive tract infections. The hygiene practices of masks like reuse, hand hygiene, prolonged wear, and washing play an important role.⁶

There are limited studies on the type of microorganisms isolated from used masks in community settings, hence this study was undertaken to detect microorganisms from used face masks in the community during the respiratory viral pandemic.

Materials and Method

This study was an observational study conducted from June to September of 2021 in the Department of Microbiology, ABVIMS and Dr Ram Manohar Lohia Hospital, New Delhi. A questionnaire was predesigned and pretested. The used masks of 47 apparently healthy individuals from

the community were collected. Masks used by in-patient individuals were excluded from the study. For uniformity, the investigators were trained in eliciting history for filling up the questionnaire, observations to be made while collecting the used face masks and collection, storage and transportation of masks. The predesigned pretested questionnaire was filled out for each participant. The used face mask was collected and stored in a new zip-lock bag which was sealed and properly labelled. A new cloth mask was given to each individual for use to avoid the inconvenience. This study was conducted for public education. Ethical approval from authorities and informed consent from participants were not required.

Bacterial and Fungal Culture

For the isolation of bacteria, a square inch area was cut from the central portion of the mask and placed on blood agar (BA) and MacConkey agar. The plates were incubated overnight at 37°C in aerobic conditions. Preliminary identification of the colony was done by performing a Gram stain and catalase test from the culture colonies; final identification was done using an automated VITEK-2 system.

For fungal culture, swabbing of the inner and outer layers of the mask was done. Four Sabouraud dextrose agar (SDA) slants were inoculated. Two slants were incubated at 25°C and others at 37°C. Following the growth of the fungi, the lactophenol cotton blue (LPCB) mount was prepared and examined microscopically. The complete process is shown in Figure 1.

Viral RNA Extraction and Molecular Detection

Specimens for COVID-19 PCR were collected by swabbing the inside and outside of the mask and transferring the swab in viral transport media (VTM). QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) was used for nucleic acid extraction as per the manufacturer's instructions and the ICMR-approved RT-PCR kit was used for the detection of the SARS-CoV-2 genes as per the recommended protocol.⁷

Statistical Analysis

The data collected was entered into a Microsoft Excel sheet and was cleaned before subjecting to analysis. Simple descriptive tabulations were drawn. The quantitative variables were mentioned as percentages and proportions. To test associations between qualitative variables, chi-square test was used. A p value of < 0.05 was considered significant.

Results

Evaluation of Mask Use and Hygiene

Forty-seven used face masks were collected from individuals of various occupations like drivers (13, 27.66%), hawkers (9, 19.15%), maids (10, 21.28%) and healthcare workers

(HCWs) (15, 31.91%). HCWs constituted the non-medical personnel working in and around healthcare vicinity like security guards, safai karamcharis, office staffs, etc. A majority of the individuals were found to be using cloth masks (23, 48.94%) followed by the ones using surgical masks (13, 27.66%) and N95 masks (11, 23.40%). On observation, 30 (63.83%) individuals were found to be wearing their masks properly covering the nose, mouth and chin. The remaining (17, 36.17%) were found to be wearing their masks without covering the nose or hanging below their chin. On comparing the occupations to proper

wearing of masks, it was observed that the odds of wearing masks properly was 5.73. It was higher among the HCWs as compared to the others combined (Table 1).

The study population was divided based on the duration of use of masks (in hours) (Table 2). The majority (36, 76.60%) were found to be wearing their masks for ≥ 6 hours with isolates consisting of 71 (76.34%) bacterial and 30 (81.08%) fungal isolates. Two participants wore the masks for ≤ 2 hours where 4 (4.30%) bacteria and 2 (5.41%) fungi were isolated. Nine participants wore the masks for 3–5 hours with 18 (19.35%) bacterial and 5 (13.51%) fungal isolates.

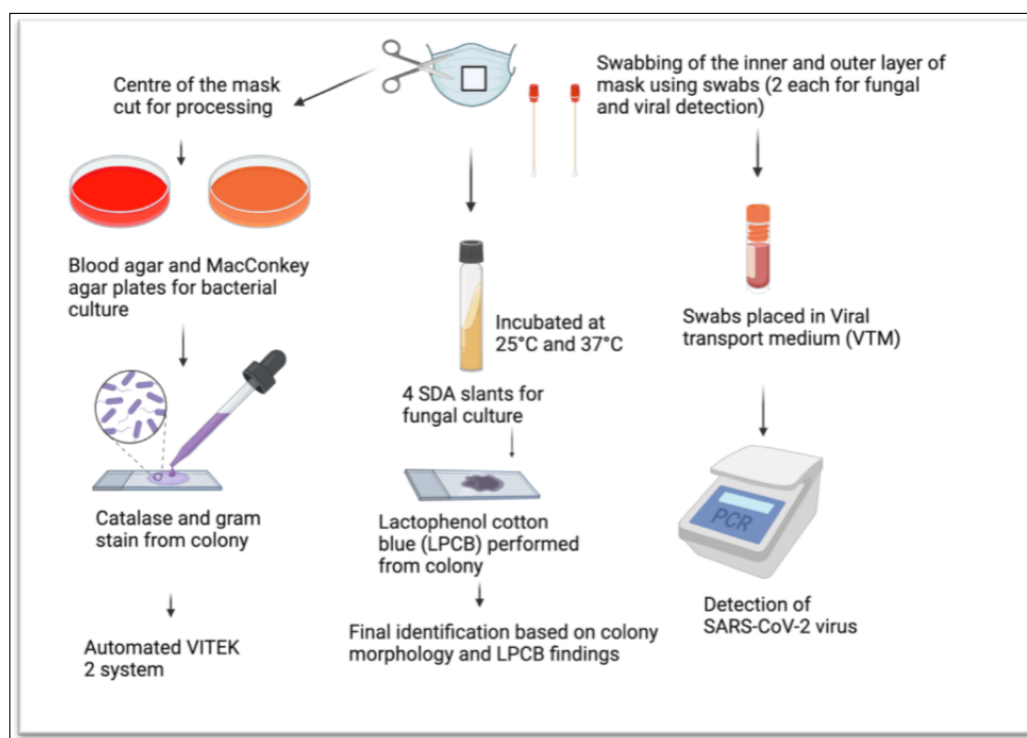


Figure 1. Method used for Microbial Detection (Created in BioRender.com)

Table 1. Observation of Masks Worn by Individuals from Different Occupations

Proper Wearing of Masks	Yes	No
Drivers/ hawkers/ maids	17	15
Healthcare workers	13	2

p value = 0.025 (significant)

Table 2. Frequency of Bacteria and Fungal Isolates based on Duration of Wear (in Hours)

Duration of Wear (Hours)	No. of Individuals using Masks n (%)	Bacterial Isolates		Fungal Isolates		Total	
		Inner (n) (A)	Outer (n) (B)	Inner (n) (C)	Outer (n) (D)	Bacteria (A + B) n (%)	Fungal (C + D) n (%)
≤ 2	2 (4.25)	ASB (2),	ASB (1), <i>Klebsiella pneumoniae</i> (1)	<i>As. flavus</i> (1)	<i>As. niger</i> (1)	4 (4.30)	2 (5.41)

3–5	9 (19.15)	CoNS (5), ASB (3), <i>Pseudomonas</i> (1)	CoNS (2), ASB (3), Diphtheroids (2), <i>Klebsiella pneumoniae</i> (1), <i>Pseudomonas</i> (1)	<i>As. niger</i> (1)	<i>Alternaria</i> (1) <i>Fusarium</i> (2) <i>As. niger</i> (1)	18 (19.35)	5 (13.51)
≥ 6	36 (76.60)	ASB (8), CoNS (17), Diphtheroids (3), <i>Klebsiella pneumoniae</i> (1), <i>Micrococcus</i> (6), <i>Staphylococcus aureus</i> (1)	ASB (14), CoNS (13), Diphtheroids (3), <i>Enterobacter cloacae</i> (1), <i>Klebsiella pneumoniae</i> (1), <i>Micrococcus</i> (3)	<i>As. niger</i> (5), <i>As. flavus</i> (1), <i>As. glaucus</i> (1), <i>Alternaria</i> (3), <i>Penicillium</i> (1), <i>Rhizopus</i> (2), <i>Scedosporium</i> (1), <i>Chaetomium</i> (1)	<i>As. niger</i> (4), <i>As. glaucus</i> (1), <i>As. nidulans</i> (1), <i>As. flavus</i> (1), <i>Alternaria</i> (4), <i>Bipolaris</i> (1), <i>Penicillium</i> (1), <i>Rhizopus</i> (1), <i>Scedosporium</i> (1)	71 (76.34)	30 (81.08)
Total	47 (100.00)	47 (50.54)	46 (49.46)	17 (45.95)	20 (54.05)	-	-

ASB: Aerobic Spore-bearing Bacilli, CoNS: Coagulase-Negative Staphylococcus

For bacterial isolation from the masks' surfaces, as per the observations in Table 3, it was seen that the proportion of masks that showed culture positivity showed a rise of 4.2-fold from ≤ 2 hours to 3–5 hours of usage of masks and approximately 4-fold rise from 3–5 hours to ≥ 6 hours of usage of masks. Similarly, considering the fungal isolates, there was a 2.5-fold rise from ≤ 2 hours to 3–5 hours and a nearly 6-fold rise from 3–5 hours to ≥ 6 hours of usage of masks.

Table 3. Relation of Number of Bacterial and Fungal Isolates Obtained from Inner and Outer Surfaces of Used Masks to Duration of Wearing the Masks

Duration of Wear (Hours)	Bacterial Isolates n (%)	Fungal Isolates n (%)
≤ 2	4 (4.25)	2 (2.12)
3–5	18 (19.14)	5 (5.32)
≥ 6	71 (75.50)	30 (31.91)

Microbial Isolation from the Used Face Masks

The microorganisms isolated from used cloth masks, surgical masks, and N95 masks were 69 (53.07%), 32

(24.62%) and 29 (22.31%) respectively (Table 4). A total of 93 bacterial isolates were seen among which coagulase-negative *Staphylococcus* (CoNS) constituted 37 (39.78%), followed by Aerobic Spore-bearing Bacilli (ASB) (31, 33.33%). Gram-negative organisms of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* accounted for 7.53% of the total isolates. The number of bacterial isolates from the inner (47, 50.54%) and outer surfaces (46, 49.46%) of the masks did not show any significant difference (p value = 0.883). Similarly, the fungal isolates from the outer surface (20, 54.05%) and the inner surface (17, 45.95%) of used masks did not show any significant difference (p value = 0.485).

Thirty-seven fungi were isolated from the used cloth masks. A majority (48.64%) of the fungal isolates were *Aspergillus* species (*As. niger*, *As. flavus*, *As. nidulans*, *As. glaucus*). The other fungal isolates were *Alternaria*, *Rhizopus*, *Fusarium*, *Penicillium*, *Scedosporium*, *Chaetomium* and *Bipolaris*.

Bacterial growth was observed in all the masks while fungal growth was seen in 26 (55.32%) out of the total 47 masks. Of the 26 showing fungal growth, 11 (42.31%) depicted poly-fungal growth and 15 (57.69%) showed mono-fungal growth. No SARS-CoV-2 RNA was detected in any of the masks.

Table 4. Profile of Microbial Isolates

Isolates	Cloth Mask (n)	Surgical Mask (n)	N95 Mask (n)	Total n (%)
Bacterial				
ASB	13	6	12	31 (33.33)
CoNS	22	10	5	37 (39.78)
Diphtheroids	3	3	2	8 (8.60)
<i>Micrococcus</i>	3	5	1	9 (9.68)
<i>Klebsiella pneumoniae</i>	1	1	2	4 (4.30)
<i>Pseudomonas aeruginosa</i>	2	0	0	2 (2.15)
<i>Enterobacter cloacae</i>	1	0	0	1 (1.08)
<i>Staphylococcus aureus</i>	0	1	0	1 (1.08)
Fungal				
<i>As. niger</i>	9	1	2	12 (32.43)
<i>As. flavus</i>	1	1	1	3 (8.10)
<i>As. glaucus</i>	1	1	0	2 (5.40)
<i>As. nidulans</i>	0	1	0	1 (2.70)
<i>Alternaria</i>	4	2	2	8 (21.62)
<i>Bipolaris</i>	1	0	0	1 (2.70)
<i>Scedosporium</i>	2	0	0	2 (5.40)
<i>Penicillium</i>	2	0	0	2 (5.40)
<i>Rhizopus</i>	3	0	0	3 (8.10)
<i>Fusarium</i>	0	0	2	2 (5.40)
<i>Chaetomium</i>	1	0	0	1 (2.70)
Total bacterial and fungal isolates N (%)	69 (53.07)	32 (24.62)	29 (22.31)	130 (100.00)

n: Number of isolates, ASB: Aerobic Spore-bearing Bacilli, CoNS: Coagulase-Negative Staphylococcus

Discussion

The majority of our study population used cloth masks (48.94%) followed by surgical masks (27.66%) and N95 masks (23.40%). This is in concordance with a survey conducted by Selvaranjan et al. where the maximum population (40%) was using cloth masks and only 9% were using N95 masks.⁸ Use of cloth masks in the community was recommended and strongly promoted by national and international authorities.^{1,2} The high usage of cloth masks in the current study could be due to the availability of affordable and low-cost cloth masks.

Sixty-nine (53.07%) microbial organisms were isolated from cloth masks comprising a majority of the isolates. Cotton absorbs moisture and hence provides a suitable environment for microbial growth if not washed daily or washed inadequately.⁹ The high isolation rate of organisms in cloth masks could be due to inadequate

washing and/ or drying or storing of masks. The favourable microenvironment can support the accumulation and multiplication of various organisms with the potential to be pathogenic depending on the immune status of the individuals. On close observation, masks were mostly worn properly by non-medical individuals (HCWs) working in the hospital as compared to the other study groups (drivers, hawkers, maids). This could be due to more access to training and awareness within the hospital premises on hygiene etiquette and proper usage of masks.

CoNS was the most isolated bacteria constituting 37 (39.78%) of total bacterial isolates. Our finding was similar to that of Yousefimashouf et al.¹⁰ The healthy skin of humans is colonised by different CoNS at different ratios depending on the body site. *S. epidermidis*, *S. hominis*, *S. haemolyticus*, and *S. lugdunensis* are some of the common commensals from the skin. CoNS like other skin commensals could easily adhere to the surface of the mask that is in direct contact

with skin or transmitted from the hands on touching the outer layer of the mask.⁶ In healthcare settings, colonisation by CoNS on medical devices and hospital surfaces is a huge burden and is a commonly reported source of device-associated infections as reported by Maleki et al. Another possible commensal was ASB constituting 31 (33.33%) bacterial isolates.¹¹ Possible ASB in this present study could be *Bacillus* species that was reported to be commonly isolated from face masks in other studies.^{6,12,13} However, final identification of the ASB was not possible in our study due to resource constraints. Other gram-positive isolates were diphtheroids and *Micrococcus* with a combined rate of 9.68% from the total bacterial isolates.

Our study isolated only one *Staphylococcus aureus* from a surgical mask that had been worn for more than 6 hours. *Staphylococcus aureus* are transient colonisers of anterior nares in 20%–40% of healthy individuals and are found comparatively at a lower rate in human skin.⁵ However, it can cause a variety of infections like respiratory infections, and skin and soft tissue infections in immunocompromised individuals or when the normal skin barrier is affected. *Staphylococcus aureus* was a commonly reported secondary bacterial complication (76.5%) in COVID-19 patients based on analysis of various literature.¹⁴

Gram-negative bacilli (GNB) isolation rate was 7 (7.53%) out of a total of 93 bacterial isolates with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* contributing 4.30%, 2.15% and 1.08%, respectively. A mask study on healthcare personnel by Nightingale et al. revealed *Klebsiella pneumoniae* (14.5%) as the major GNB isolate followed by *Enterobacter* spp. (13.0%).¹⁵ The isolation of GNB could be due to unhygienic practices like improper storage of masks in between use and repeated touching of masks, thereby resulting in the transfer of GNB from environmental surfaces to the masks.^{3,5}

A majority (36, 76.60%) of our study participants used their masks for a duration of ≥ 6 hours. Seventy-one (76.34%) bacteria and 30 (81.08%) fungi were isolated from face masks worn for ≥ 6 hours. As depicted in Table 3, there was an exponential increase in the isolates from the used masks. This has also been seen in experimental studies where the number of pathogens isolates increased substantially over 2 hours of wear.^{16,17} Transfer of commensal flora from the skin and upper respiratory tract to the inner surface of the mask could be one plausible answer. The isolates from the outer surface could be mixed bacteria and spores present in the air. Their presence could also be due to the unintentional transfer of the commensal flora from the hands of the users.¹⁰

Among the 47 masks that were under evaluation, 21 (44.68%) resulted in no fungal growth, 15 (31.91%) had only one type of fungal isolate, and 11 (23.40%) showed

poly-fungal growth. Based on the colony morphology and microscopic examination of the spores and hyphal structures, 27 fungi were identified. Eight fungal genera were identified, namely *Aspergillus*, *Alternaria*, *Bipolaris*, *Chaetomium*, *Fusarium*, *Penicillium*, *Rhizopus* and *Scedosporium*. *Aspergillus* species (48.65%) was the most common fungal isolate in our study (Table 2). Our finding was similar to the results of the study of Keri et al. where *Aspergillus* species were the predominant fungal isolates.¹⁸ Fungal spores are widespread in the environment and fungal contamination of masks is not surprising. One notable finding in our study was the isolation of *Rhizopus* in masks that were worn for more than 6 hours duration. *Rhizopus* is usually an opportunistic fungal infection in immunocompromised patients. Mucormycosis is a rare but fatal infection, a high number of cases of which, were reported in India during the pandemic. A systemic review of various studies reported nose and sinus (88.9%) to be the most common site affected followed by rhino-orbital (56.7%).¹⁹ Though it is thought to be an opportunistic infection for immunocompromised individuals, a few cases of mucormycosis in immunocompetent people have also been reported.^{20,21}

A study by MacIntyre et al. confirmed respiratory illnesses and laboratory-confirmed viruses to be significantly higher among cloth mask users.²² The transmission of SARS-CoV-2 virus is reported to be through direct contact, droplet inhalation or airborne transmission. However, the rate of acquisition of SARS-CoV-2 virus through surface transmission is difficult to prove with a few possible cases being reported.^{23,24} The present study did not detect any SARS-CoV-2 virus in our study masks as the masks were collected from apparently healthy individuals while they were at work. Studies have been conducted on the viability of the SARS-CoV-2 virus on porous and non-porous surfaces. As per previous research, the viability on porous and non-porous surfaces lasts for minutes to hours and days to weeks respectively.^{25–27} This could be another probable reason for our current study finding on the SARS-CoV-2 virus in our study masks. Other respiratory viral pathogens detection was not performed due to limited resources.

The limitation that needs to be addressed is the small sample size. The undesirable outcomes from wearing masks were not assessed creating a gap in our study. Further studies and more evidence-based research on mask hygiene are required.

Conclusion

The importance of using face masks for the prevention of infection transmitted through the respiratory route cannot be overemphasised. In our observational study, a majority of the HCWs used the mask properly due to increased awareness and training provided to them. Hence,

strengthening the guidelines on hygiene etiquette and proper training becomes essential. It was also evident that skin flora and environmental commensals colonised the mask. Along with bacteria, fungus was also found colonising the mask. We observed 3 isolates of *Rhizopus* on face masks. Additionally, microorganisms accumulated on the face masks after 2 hours of wear and exponentially increased in number as the duration of wear increased. These findings would be helpful in recommending the duration of use of masks and the importance of following proper hygiene etiquette.

Source of Funding: None

Conflict of Interest: None

References

1. World Health Organization [Internet]. Advice on the use of masks in the context of COVID-19: interim guidance, 5 June 2020; 2020 [cited 2023 Jun 19]. Available from: <https://apps.who.int/iris/handle/10665/332293> [Google Scholar]
2. Ministry of Health and Family Welfare, Government of India [Internet]. Additional guidelines on rational use of personal protective equipment (setting approach for health functionaries working in non-COVID areas); 2020 [cited 2023 Jun 22]. Available from: https://www.mohfw.gov.in/pdf/Additional_guidelines_on_rational_use_of_Personal_Protective_Equipment_setting_approach_for_Health_functionaries_working_in_non_COVID_areas.pdf
3. Kisielinski K, Giboni P, Prescher A, Klosterhalfen B, Graessel D, Funken S, Kempfski O, Hirsch O. Is a mask that covers the mouth and nose free from undesirable side effects in everyday use and free of potential hazards? *Int J Environ Res Public Health*. 2021;18(8):4344. [PubMed] [Google Scholar]
4. Chiller K, Selkin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. *J Invest Dermatol Symp Proc*. 2001;6(3):170-4. [PubMed] [Google Scholar]
5. Parlet CP, Brown MM, Horswill AR. Commensal *Staphylococci* influence *Staphylococcus aureus* skin colonization and disease. *Trends Microbiol*. 2019;27(6):497-507. [PubMed] [Google Scholar]
6. Delanghe L, Cauwenberghs E, Spacova I, Boeck ID, Beeck WV, Pepermans K, Claes I, Vandenheuvel D, Verhoeven V, Lebeer S. Cotton and surgical face masks in community settings: bacterial contamination and face mask hygiene. *Front Med (Lausanne)*. 2021;8:732047. [PubMed] [Google Scholar]
7. ICMR-National Institute of Virology [Internet]. Standard operating procedure for detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by rRT-PCR : confirmation assay; 2020 [cited 2023 Jul 5]. Available from: https://www.icmr.gov.in/pdf/covid/labs/2_SOP_for_Confirmatory_Assay_for_2019_nCoV.pdf
8. Selvaranajn K, Navaratnam S, Rajeev P, Ravintherakumaran N. Environmental challenges induced by extensive use of face masks during COVID-19: a review and potential solutions. *Environ Chall*. 2021;3:100039. [Google Scholar]
9. Chughtai AA, Seale H, Macintyre CR. Effectiveness of cloth masks for protection against severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis*. 2020;26(10):e200948. [PubMed] [Google Scholar]
10. Yousefimashouf M, Yousefimashouf R, Alikhani MS, Hashemi H, Karami P, Rahimi Z, Hosseini SM. Evaluation of the bacterial contamination of face masks worn by personnel in a center of COVID 19 hospitalized patients: a cross-sectional study. *New Microbes New Infect*. 2023;52:101090. [PubMed] [Google Scholar]
11. Maleki A, Shamshirgaran M, Aramjoo H, HajiHosseini M, Moghadam HM, Bidaki MZ. An evaluation of frequencies and types of bacterial pathogens in personnel gowns, before and after washing with sodium hypochlorite 5%, in Vali-e-Asr Hospital, Birjand, Iran. *J Surg Trauma*. 2019;7(3):98-102. [Google Scholar]
12. Park AM, Khadka S, Sato F, Omura S, Fujita M, Hashiwaki K, Tsunoda I. Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Sci Rep*. 2022;12(1):11361. [PubMed] [Google Scholar]
13. Lee EJ, Park H, Je MA, Jung S, Myoung G, Jo SB, Hwang HM, Si R, Jin H, Lee KE, Kim J. Microbial contamination according to the numbers of mask worn in the community. *Biomed Sci Lett*. 2022;28(4):317-21. [Google Scholar]
14. Adalbert JR, Varshney K, Tobin R, Pajaro R. Clinical outcomes in patients co-infected with COVID-19 and *Staphylococcus aureus*: a scoping review. *BMC Infect Dis*. 2021;21(1):985. [PubMed] [Google Scholar]
15. Nightingale M, Mody M, Rickard AH, Cassone M. Bacterial contamination on used face masks among nursing home healthcare personnel. *Antimicrob Steward Healthc Epidemiol*. 2023;3(1):e54. [PubMed] [Google Scholar]
16. Chughtai AA, Stelzer-Braid S, Rawlinson W, Pontivivo G, Wang Q, Pan Y, Zhang D, Zhang Y, Li L, MacIntyre CR. Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers. *BMC Infect Dis*. 2019;19(1):491. [PubMed] [Google Scholar]
17. Zhiqing L, Yongyun C, Wenxiang C, Mengning Y, Yuanqing M, Zhenan Z, Haishan W, Jie Z, Kerong D, Huiwu L, Fengxiang L, Zanjing Z. Surgical masks as source of bacterial contamination during operative procedures. *J Orthop Translat*. 2018;14:57-62. [PubMed] [Google Scholar]

18. Keri VC, Kumar A, Singh G, Mandal A, Ali H, Ranjan P, Wig N. Pilot study on burden of fungal contamination in face masks: need for better mask hygiene in the COVID-19 era. *Infez Med.* 2021;29(4):557-61. [PubMed] [Google Scholar]
19. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. *Diabetes Metab Syndr.* 2021;15(4):102146. [PubMed] [Google Scholar]
20. Venkatesh D, Dandagi S, Chandrappa PR, Hema KN. Mucormycosis in immunocompetent patient resulting in extensive maxillary sequestration. *J Oral Maxillofac Pathol.* 2018;22(1):S112-6. [PubMed] [Google Scholar]
21. He J, Sheng G, Yue H, Zhang F, Zhang HL. Isolated pulmonary mucormycosis in an immunocompetent patient: a case report and systematic review of the literature. *BMC Pulm Med.* 2021;21(1):138. [PubMed] [Google Scholar]
22. MacIntyre CR, Seale H, Dung TC, Hien NT, Nga PT, Chughtai AA, Rahman B, Dwyer DE, Wang Q. A cluster randomised trial of cloth masks compared with medical masks in healthcare workers. *BMJ Open.* 2015;5(4):e006577. [PubMed] [Google Scholar]
23. Meyerowitz EA, Richterman A, Gandhi RT, Sax PE. Transmission of SARS-CoV-2: a review of viral, host, and environmental factors. *Ann Intern Med.* 2021;174(1):69- 79. [PubMed] [Google Scholar]
24. Kampf G, Bruggemann Y, Kaba HE, Steinmann J, Pfaender S, Scheithauer S, Steinmann E. Potential sources, modes of transmission and effectiveness of prevention measures against SARS-CoV-2. *J Hosp Infect.* 2020;106(4):678-97. [PubMed] [Google Scholar]
25. Chatterjee S, Murallidharan JS, Agrawal A, Bhardwaj R. Why coronavirus survives longer on impermeable than porous surfaces. *Phys Fluids (1994).* 2021;33(2):021701. [PubMed] [Google Scholar]
26. Chin AW, Chu JT, Perera MR, Hui KP, Yen HL, Chan MC, Peiris M, Poon LM. Stability of SARS-CoV-2 in different environmental conditions. *Lancet Microbe.* 2020;1(1):e10. [PubMed] [Google Scholar]
27. Liu Y, Li T, Deng Y, Liu S, Zhang D, Li H, Wang X, Jia L, Han J, Bei Z, Li L, Li J. Stability of SARS-CoV-2 on environmental surfaces and in human excreta. *J Hosp Infect.* 2021;107:105-7. [PubMed] [Google Scholar]