Microbial load and proliferation associated with various face mask types and sources during the COVID-19 pandemic

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ABSTRACT: Due to the shortage of personal protective equipment during the COVID-19 pandemic, homemade face coverings were recommended as alternatives. However, the capability of alternative face coverings to proliferate microbes have not been fully documented.

The current study evaluated bacterial load and proliferation associated with the use of common face masks during the COVID-19 pandemic. Mask type-specific and surface-related bacterial load and pattern were noticeable in the study. Results indicated that roadside masks are among samples that contained relatively higher initial bacterial load. The highest number of bacterial forming colonies were observed in the inner surface of mask samples. Proliferation of microbes over time was also noticeable among the non-certified face coverings included in the study. Sterilization or washing of non-certified fabric face masks before use is recommended.

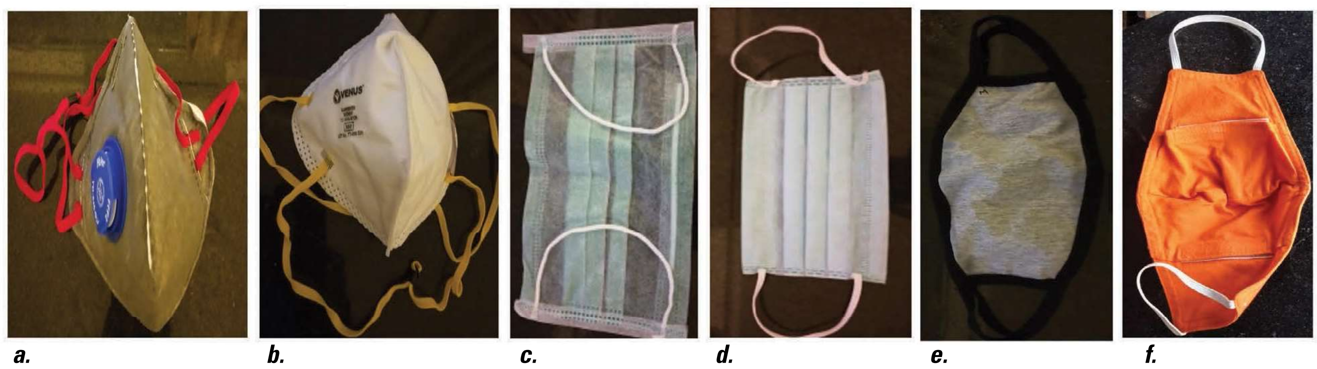
Application: Apart from protecting the wearer from getting exposed to the infection, face coverings also stop the spread from infected individuals. The present study examined the population of microbes distributed over the mask surface during use. The microbial load on each type of mask, the health, and potential safety risks were highlighted. This information could guide policy and mitigating measures against severe acute respiratory syndrome (SARS) related infections.

Studies have shown that the use of face masks can minimize the spread of COVID-19 [1,2]. Masks provide a physical barrier between the outside environment and the respiratory organs of the user [3]. Filtration efficiency is one of the major factors in the evaluation of the effectiveness of face masks. Face masks that provide the highest bacterial filtration and high resistance to body fluids are categorized as Level 3 by the American Society for Testing and Materials (ASTM) Standard [4]. In response to the shortage of face masks during the COVID-19 pandemic, the use of homemade masks designed from regular fabrics was recommended by the U.S. Centers for Disease Control and Prevention [5]. The shortage is expected to persist, and the World Health Organization has estimated that approximately 89 million medical masks will be needed every month during the COVID-19 pandemic [6].

As most countries lift lockdowns, individuals are required to wear face masks, especially in public [1], resulting in greater demand for masks to meet the increasing needs [7]. The use of homemade cloth face coverings and reuse of disposable face masks have become a common habit due to a critical shortage in supply of masks during the COVID-19 pandemic [8]. The global price of face masks significantly increased during the pandemic [9,10]. Between January and February 2020, the price of face masks increased by 319% in the United States [10]. Because of a large customer base, Asia Pacific became the largest market for face coverings at the onset of the COVID-19 pandemic, accounting for 33.7% of global market share in 2019. India and China are among the top manufacturing countries of face masks in the world [11].

For these reasons, non-certified and homemade masks were designed, manufactured [7], and sold in open markets [12]. In most cases, the purchased masks fall below the required health standards. In developing countries, a low level of income has been cited as another major reason for low-quality manufacturing of face masks, because many people cannot afford protective masks that meet required health standards or certifications [12]. Most users of face masks made from regular fabrics (roadside tailors) use them without washing and are prone to contracting other diseases besides COVID-19 [13].

Bacterial dispersion has been associated with the use of face masks [14,15], and such dispersion may be influenced by the design of the face mask [16]. Aerosol filtration performance of household fabrics and surgical masks and their effectiveness at blocking droplets have been investigated [5,8,17,18,19], but their capability to proliferate microbes during use has not been fully documented. The goal of the current study is to evaluate bacterial load and proliferation associated with the use of face masks during the COVID-19 pandemic. In the study, we (1) quantified initial bacterial loads on face masks of differ-



1. Images of face covering sample types utilized in the current study: (a) N95 mask with filter (FiltAir-FFP2); (b) N95 mask without filter (VENUS: V-4400N95 National Institute for Occupational Safety and Health [NIOSH]); (c) two-layered (two-ply) surgical disposable face mask; (d) three-layered (28 polypropylene [PP] + 20 meltblown [MB] + 20 PP) mask; (e) roadside mask (baniyan cloth); and (f) Velcro face mask (cotton fabric).

ent types and sources, and (2) examined their proliferation on the inner and outer mask surfaces during use. Masks with varying qualities and prices were utilized.

EXPERIMENTAL

Materials and methods

N95 masks (VENUS: V-4400N95 National Institute for Occupational Safety and Health [NIOSH]) with a filter (FiltAir-FFP2) and without a filter were purchased from New Venkateswara Medicals (Chennai, India). Two-layered and three-layered polypropylene masks were purchased from a retailer in Chennai. Velcro face masks were purchased from a retailer in Bengaluru. Roadside masks (baniyan cloth) were purchased from a market in Chennai, India. **Figures 1a-1f** shows images of the masks used in this study.

Soyabean casein digest agar was purchased from HIMEDIA (Mumbai, India) and prepared according to the manufacturer's specifications. Briefly, 40 g of the agar was suspended in 1 L of distilled water in a 2 L volumetric flask followed by thorough mixing to obtain complete dissolution. The suspension was sterilized in an autoclave (Raagaa

		Volunteers					
Masks	**Surfaces**	**Control (A)**	**(B)**	**(C)**	**(D)**	**(E)**	**(F)**
N95 (with filter)	Inside	1	65	166	136	NA	123
	Outside	2	244	313	65	NA	140
N95 (no filter)	Inside	3	195	163	153	245	121
	Outside	0	89	257	58	52	9
Two-layered	Inside	1	439	107	91	347	163
	Outside	2	110	158	12	10	19
Three-layered	Inside	29	453	263	840	529	577
	Outside	38	515	200	320	9	35
Roadside	Inside	26	835	457	602	387	635
	Outside	46	200	213	19	44	18
Velcro	Inside	2	430	471	455	145	371
	Outside	10	86	188	40	9	18
NA = N95 masks (with and without filters) missed by volunteer E.							

I. Viable microbial load observed within the internal and external surfaces of different face masks. Number of bacterial colonies (colony forming units; CFUs) is presented.

Volunteers							
Masks	Surfaces	Control (A)	(B)	(C)	(D)	(E)	(F)
N95 (with filter)	Inside	0	0	0	1	NA	1
	Outside	0	0	3	5	NA	2
N95 (no filter)	Inside	0	0	0	2	2	1
	Outside	0	0	0	6	1	4
Two-layered	Inside	0	0	0	1	1	0
	Outside	0	0	0	4	3	1
Three-layered	Inside	0	0	0	1	2	0
	Outside	0	0	0	1	0	0
Roadside	Inside	0	0	0	0	0	0
	Outside	0	0	0	2	1	2
Velcro	Inside	0	0	0	0	0	0
	Outside	0	0	1	1	1	0
NA = N95 masks (with and without filters) missed by volunteer E.							

II. Fungal colonies observed within the internal and external surfaces of different face masks. Number of bacterial colonies (CFUs) is presented.

Industries; Nanganallur, Chennai) at 120°C for 30 min. The flask was allowed to cool down and the agar media was poured into petri dishes and allowed to solidify. Sabouraud dextrose agar (SDA) was purchased from SRL Chem (Chennai, India) and prepared according to the manufacturer's specifications. Briefly, 65 g of the SDA powder was suspended in 1 L of distilled water, mixed thoroughly for complete dissolution, and sterilized in an autoclave at 120°C for 30 min. The media was allowed to cool, and the content was dispensed into petri dishes and allowed to solidify.

The study was conducted at Asthagiri Herbal Research Foundation, Chennai, India. Five volunteers who work within the premises were selected for the study. The volunteers were comprised of three females and two males with an age range of 23–68. One female and one male were diabetic. To avoid post-purchase contamination, masks remained sealed in a clean Ziploc bag immediately after purchase, and volunteers were discouraged from touching the masks or adjusting the fit during the experiments. Each participant was asked to wear the mask types for a period of 1 h per mask. All participants were non-smokers, and they consumed food and water during the study. All the volunteers remained within the premises until the end of the experiment.

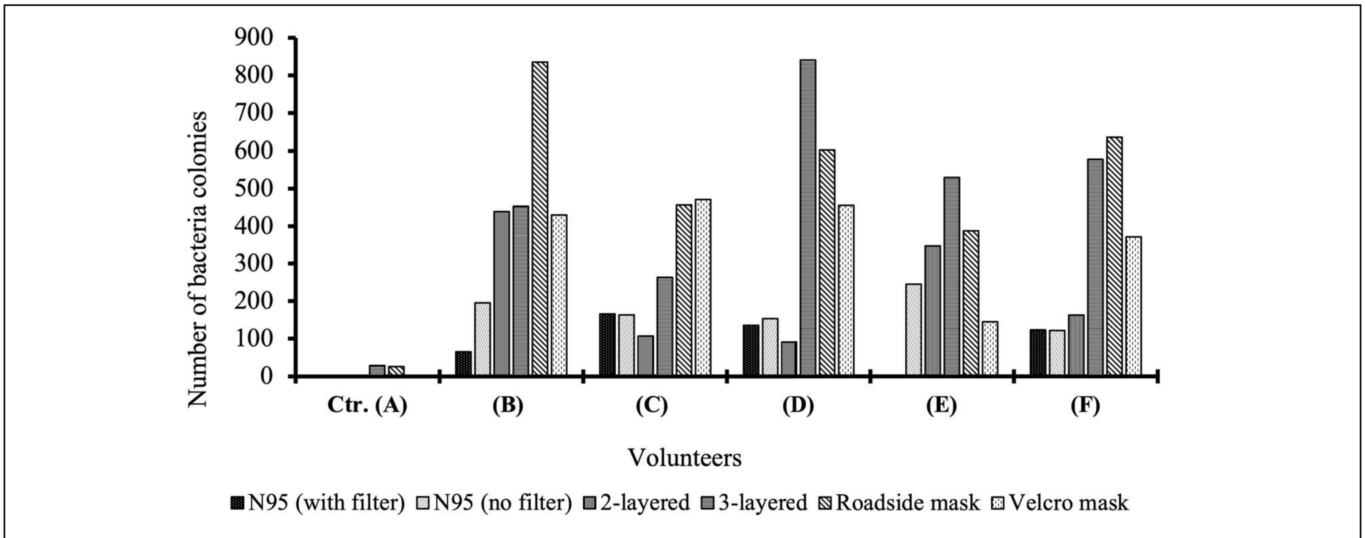
At the end of the experiment, the center part of all masks at an area of 3 mm × 8 mm was collected inside a laminar air flow chamber by making a cut using sterile surgical blades. The cut centers were immediately imprinted on the sterile soyabean casein digest agar medium in separate petri dishes. The inside and outside layer of the mask was imprinted separately. Unused masks were used as a control and were also imprinted on agar media before and after the period of the experiments. After imprints, the plates were incubated at 30°C and observed for the growth of bacterial colonies after 24 h. The incubator used was previously purchased from Elegant Equipment (Thirumullaivoyal, Chennai). The SDA medium was used to observe the fungal colonies after 5 days of incubation at 30°C.

Samples of observed bacterial colonies were diluted in 500 µL of sterile distilled water by suspending an inoculating loop full of the culture. A clean and sterile glass slide was taken and 100 µL of the suspension was placed at the center. It was fixed by gently heating the slide over a burner. A crystal violet was added and kept for about 30 s and then rinsed with water. The smear was covered with Gram's iodine for 1 min and washed with water, followed by 95% ethanol wash for about 10–20 s. Safranin was added and after 1 minute washed with water. The dried slide was observed under a Binocular Microscope (Deep Vision Plus DXL 25T, GA Instruments; Chennai, India) at 40X.

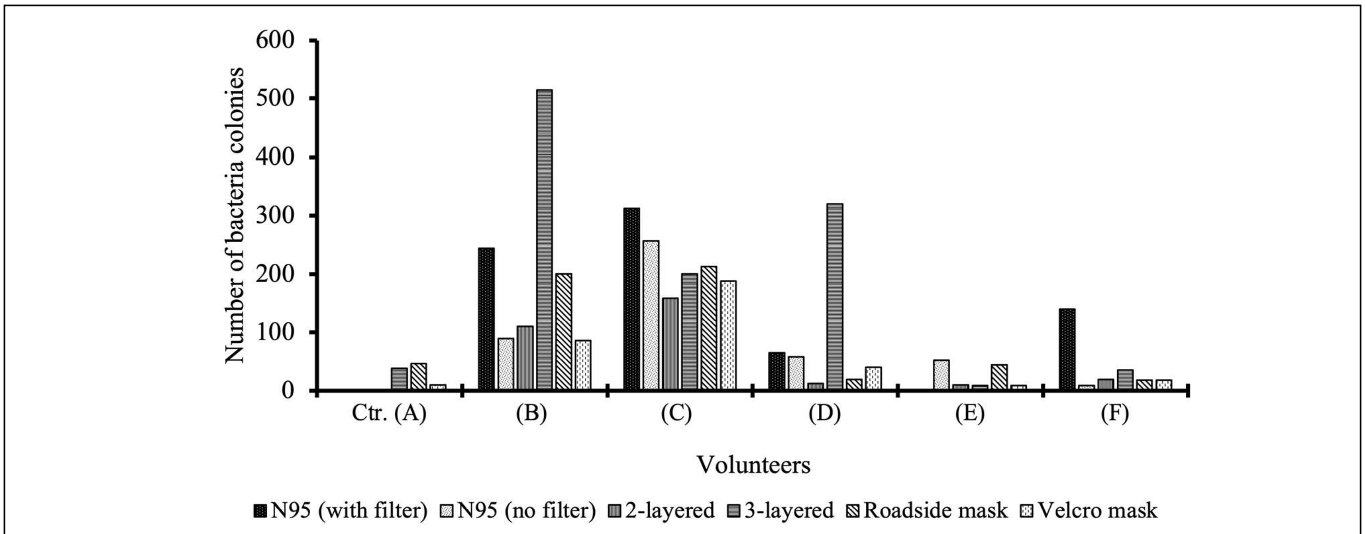
RESULTS

The results of viable microbial load on the inner and outer surfaces of face masks at the beginning (control) and the end of the experiments are given in **Tables I and II** and in **Figs. 2 and 3** as colony forming units (CFUs).

Images of face masks utilized in the study are presented



2. Bacterial loads counted as colony forming units (CFUs) obtained from the inside part of various face mask types from different sources.



3. Bacterial loads counted as CFUs obtained from the outside part of various face mask types from different sources.

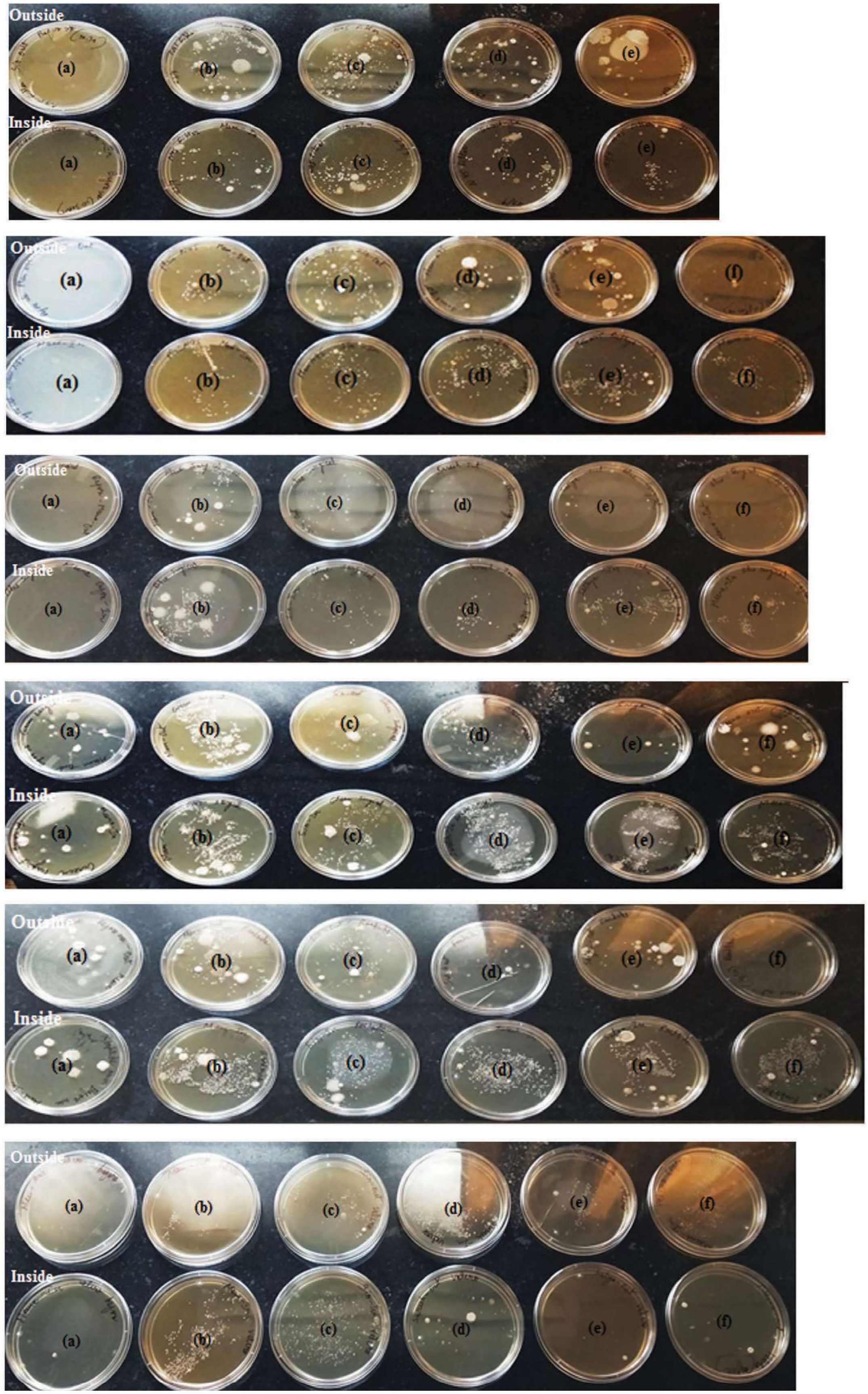
as Figs. 1a–1f. Images of petri dishes showing viable CFUs observed from samples collected from inner and outer surfaces of masks are given in **Figs. 4a–4f**.

Similar bacterial colonies were observed in the mask imprint of all the volunteers. Most of the bacterial species were cocci shaped. The outside of the mask primarily contained five repeatedly occurring colonies, three out of which were gram positive and two that were gram negative (**Figs. 5 and 6**). The inside of the mask had three repeatedly occurring colonies, one out of which was gram positive and two that were gram negative (**Fig. 7**). All the volunteers had similar bacterial load on the masks used. No unique colonies were found aside from *Streptococci sp* and *Bifidobacterium* generally observed on most masks.

DISCUSSION

It has become necessary to wear face masks during and after the COVID-19 pandemic. The universal use of face coverings has attracted interests from different disciplines to understand their effectiveness [2,16]. However, there are no data on bacterial load of various face masks, especially those sourced from uncertified outlets, including roadside markets. Additionally, it is important to understand bacteria proliferation capabilities of different face mask sources and types.

In the current study, we found that the N95 (with and without filters), two-layered face masks, and Velcro masks included in the study contained the lowest initial number of bacterial CFUs. Three-layered and roadside masks contained the highest initial number of bacteria forming units (Table I). The high initial number of bacteria colonies observed in roadside-sourced face masks is consistent with the potential risk associated with the use of uncertified substandard face masks as reported by Mwema and Nyika [12]. In the present study, both surfaces of roadside masks contained a high initial number of bacterial CFUs (Figs. 2 and 3).



a. N95 with filter.

b. N95 without filter.

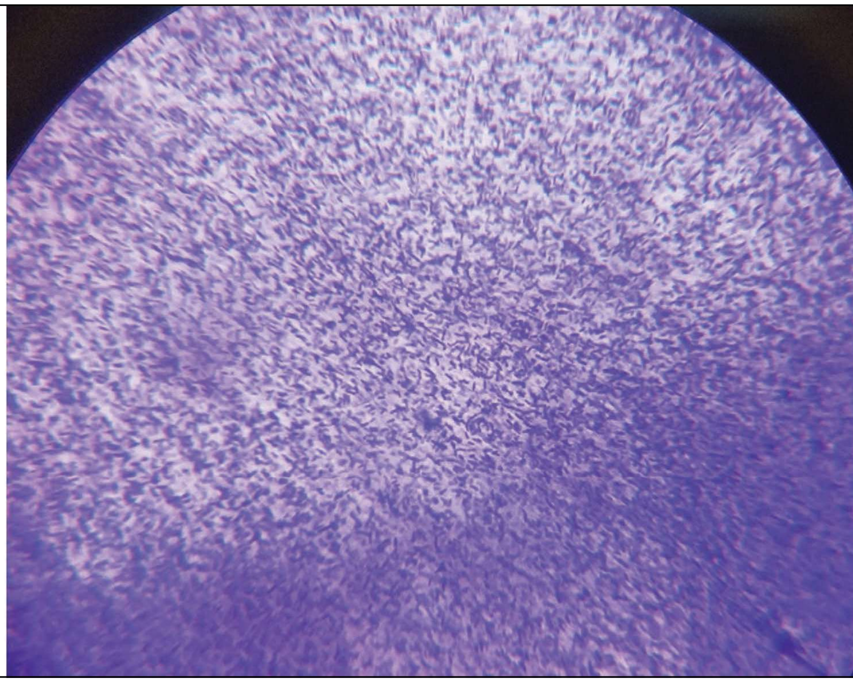
c. Two-layered mask.

d. Three-layered mask.

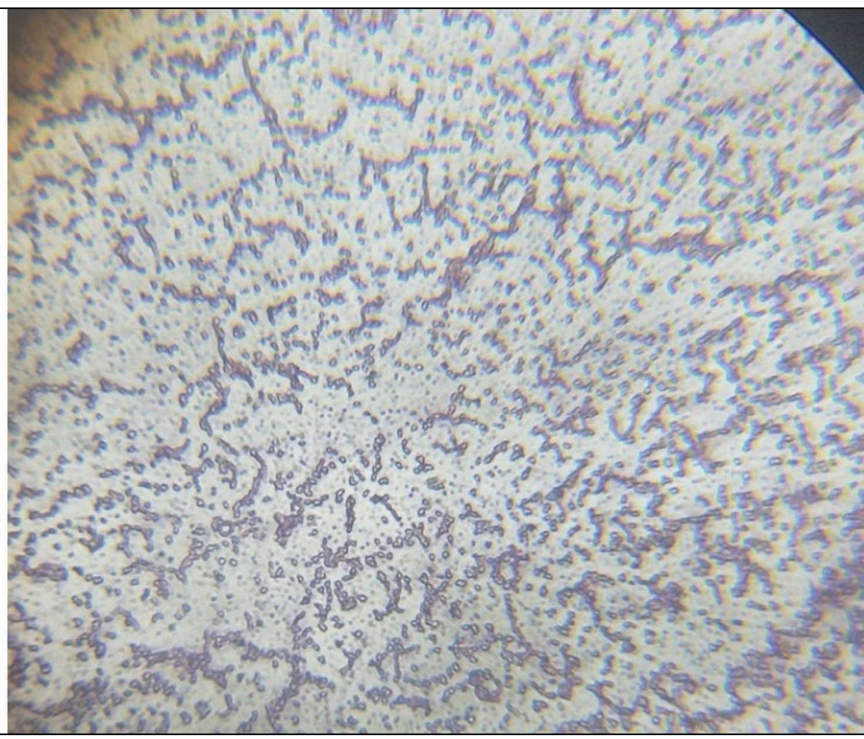
e. Roadside mask.

f. Velcro mask.

4. Post-experimental microbial colonies observed on both sides (outside and inside) of mask samples after 24 h of incubation: (a) microbial colonies in N95 mask with filter; (b) microbial colonies in N95 mask without filter; (c) microbial colonies in two-layered face mask; (d) microbial colonies in three-layered face mask; (e) microbial colonies in roadside mask; and (f) Velcro mask.

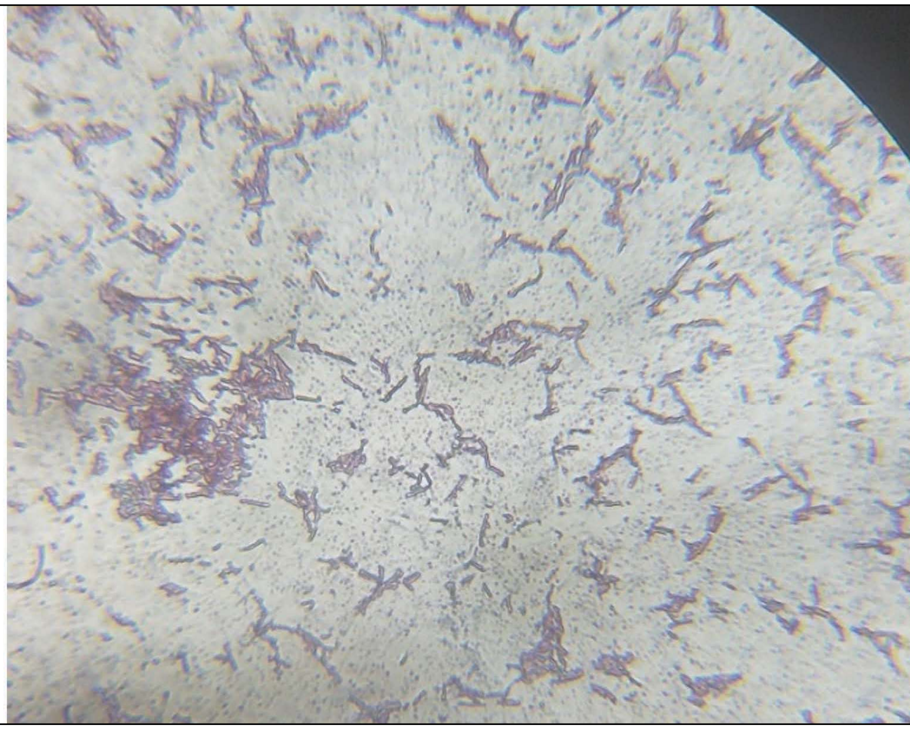


5. Gram positive **Streptococci sp** *observed on the outside of the masks.*



6. Gram negative cocci observed on the outside of the masks.

At the end of the experiments, the highest number of bacterial forming colonies were observed on the inner side of the three-layered face masks (840 CFU), followed by the inner side of the roadside face mask (835 CFU). For the outer side of the masks, the lowest CFUs were observed in N95 (without filter), three-layered, and velcro masks (9 CFU each), followed by two-layered masks (10 CFU). Bacterial proliferation with wearing time and surfaces observed in the current study is consistent with the one reported by Zhiqing et al. [20]. Face masks could proliferate bacteria with increased wearing time. Zhiqing et al. [20] demonstrated that mask users can also contribute to the source of contamination to the inner side of masks, and face masks with higher filtration could significantly reduce horizontal dispersions, resulting in the contamination of the reverse side of the mask. The higher CFUs observed in the three-layered and roadside masks at the end of the experiment may have resulted from proliferation and bidirectional hor-



7. **Bifidobacterium** *observed on the inside of the mask.*

izontal dispersion, which could be prevented or minimized by using face masks with high filtration capacity.

How microbes can survive and propagate on personal protection equipment is a factor to be considered [21]. Different patterns of proliferation and dispersion were observed in the results of the present study (Figs. 2 and 3). Cellulose-containing face mask materials are known to support the propagation of certain fungi and bacteria in ideal conditions because of their ability to digest cellulose [22]. Industrial grade polypropylene materials are recommended for the manufacturing of face masks, because they cannot serve as a nutrient for bacteria [21,23]. Differences in mask materials may have contributed to the variations in proliferation patterns observed in the current study. The material contents of roadside masks cannot be ascertained because of the absence of the manufacturer's label, which is typical for roadside face masks. Fungal load and proliferation were observed in the current study to be generally low.

The microbes associated with different face masks in the present study are ubiquitous. Streptococci species were observed in samples collected from volunteers D and E. Streptococci have been isolated from saliva samples and oral cavities [24,25]. Microorganisms of the genus Streptococci are of clinical importance because of their capability to be pathogenic [24]. They are known to cause common bacterial infections [26]. Bifidobacteria was found in samples collected from all of the volunteers. Bifidobacterium belong to the Actinobacteria phylum and are normally found in the gastrointestinal track [27]. They are considered non-pathogenic and promoted as a probiotic due to their potential beneficial effects on the gastrointestinal track. However, there have been reports implicating Bifidobacteria as pathogenic agent in various conditions such as urinary tract infections [28].

Although their classification was not a focus in the current study, the number of microbes observed on the surfaces of face coverings assessed the level of hygiene of masks available in open markets. The sources of contamination of the inner surface of the masks cannot be ascertained, but the results suggested contamination from volunteers. It should also be noted that the observed microbes proliferated differently on different mask samples. Microbial load observed on the outside surface of the masks and on controls suggests possible pre-purchase contamination.

CONCLUSIONS

Although respiratory viruses are the primary reason for the use of face coverings during the pandemic, the safety and hygiene of purchased masks in causing other diseases deserve closer attention. In this study, different types of masks from various sources, including roadside sourced masks, were examined for initial microbial load, proliferation, and possible horizontal dispersion. The initial number of bacterial CFUs was observed to be very high on both surfaces of the roadside and three-layered face masks. After wearing the masks (1 h), there was a sharp increase in the microbial load when compared to the initial load. The N95 masks with filters showed a higher number of colonies on the outside when compared to the inside, suggesting higher filtration capabilities and resistance to microbial dispersion. The microbial load was observed to be very high in roadside and three-layered face masks.

On roadside masks, the outer CFUs were lower when compared to the inside, suggesting either poor filtering capacity or material contents that support the proliferation of microbes on the inner surface and not on the outer surface. Since the masks carried some microbial population, it is suggested to sterilize or wash purchased masks before using them. Although the current study is a rapid assessment of microbial loads on common face masks sold in open markets, its results provide insights into possible prepurchase contamination of poorly designed and manufactured masks. It also highlighted health and safety risks of masks sold in open markets in causing other diseases. Sterilization or washing of fabric face masks before use is recommended. The present study was limited in the number of replications for statistical comparison. Future studies should include more elaborate and inclusive molecular studies that classify colonies up to species and subspecies levels. **TJ**

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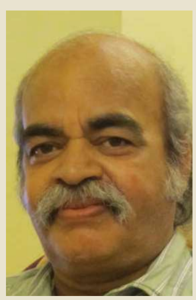

Narasimhan

Balakrishnan

Ayodeji

Ramkumar

We chose to research this topic for two reasons: (1) to explore usefulness of face masks; and (2) to assess the risk involved in continuous wearing of a mask at longer durations, resulting in increased microbial load due to filtration. This triggered our interest in knowing the effect of wearing masks that differed in cost and filtering potential; the more a mask filters, the greater likelihood of enhanced viral load and increased risk to the host.

We discovered that the use of masks carries a potential risk of enhanced viral load, which is detrimental to the host. Therefore, it is necessary to disinfect in situ. Our most interesting finding was that the microbial load is higher on the inside portion of the mask. The research showcases the importance of sterilization in offering enhanced protection.

Mask manufacturers may benefit from this information by understanding the need to develop disinfecting material and coating for cotton fabric masks. Our next step is to evaluate a non-alcoholic, nontoxic disinfecting material that can destroy a microbe on contact.

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