

# EFFICACY OF FACIAL MASK USE IN COVID-19 TRANSMISSION USING A BACTERIAL MODEL

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*The prevention of COVID-19, caused by SARS-CoV-2, involves reducing transmission of infectious respiratory droplets and aerosols with different methods, including the use of face masks. There have been a variety of studies published about the effectiveness of face masks — they have been recognised as an inexpensive yet expedient prevention method. In this research we aimed to investigate the microbial contamination of used face masks and to compare the results based on mask usage time to determine the efficiency of facial mask use against COVID-19. Microbiological contamination of 51 used face masks was analysed by the imprinting method on suitable cultivation agars and colony forming units were determined. The majority of microorganisms found in our study were those of normal human skin and respiratory tract microbiota. A greater number of bacterial species was found on the outsides of the facial masks, which furthermore increased with prolonged usage time. We conclude that face mask use is effective in both detaining excreted microorganisms as well as protecting the wearer from microbes in the air, and therefore, should be implemented in the prevention strategies of respiratory diseases. Furthermore, our results show the importance of correct facial mask usage by their contamination with a spectrum of microorganisms.*

**Keywords:** *face masks, respiratory protective equipment, COVID-19 prevention.*

## INTRODUCTION

Coronaviruses are enveloped, positive stranded RNA (+ssRNA) viruses. As the name indicates, from Latin *corona* meaning “crown”, they have a characteristic crown-like shape when viewed under an electron microscope due to the presence of spike proteins on the envelope (Masters and Perlman, 2013; Li, 2016). They are known as causative agents for respiratory and enteric, as well as hepatic and neurologic diseases in animals and humans (Corman *et al.*, 2018; Cui *et al.*, 2019). Coronaviruses belong to the *Riboviria* realm, *Nidovirales* order, *Cornidovirineae* suborder, and *Coronaviridae* family (International Committee on Taxonomy of Viruses, 2020).

In December 2019, an outbreak of pneumonia of unknown aetiology in Wuhan, China was reported (ProMED-mail,

2019). It was later found to be a novel coronavirus (Zhou *et al.*, 2020), which has had a pandemics spread around the world causing a disruption of our everyday life (Kumar *et al.* 2021). The virus, now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), belongs to coronaviruses’ *Orthocoronavirinae* subfamily’s one of four genera: *Betacoronavirus*. The full name of the species is Severe acute respiratory syndrome-related coronavirus. Other significant viruses known for causing epidemics of severe respiratory syndrome in humans from the same genera are SARS-CoV (severe acute respiratory syndrome coronavirus) and the MERS-CoV (Middle East Respiratory Syndrome Coronavirus) (Holmes and Rambaut, 2004; de Groot *et al.*, 2013; International Committee on Taxonomy of Viruses, 2020). The size of SARS-CoV-2 is described to be approximately 0.1 µm in diameter: 70–90 nm by Kim *et al.*

(2020), 66–81 nm by Park *et al.* (2020), 60–140 nm by Zhu *et al.* (2020).

The virus is thought to spread mainly via respiratory droplets and aerosols (WHO, 2020), excreted by asymptomatic or symptomatic individuals infected by the virus (Cascella *et al.*, 2022). The release of variably sized respiratory droplets possibly containing infectious agents can happen during breathing, speaking, coughing, and sneezing (Asadi *et al.*, 2019; Alsvéd *et al.*, 2020; Echternac *et al.*, 2020, Edwards *et al.*, 2021).

Respiratory droplets are usually divided into large droplets (< 5 µm in diameter) that fall to the ground after a brief period, and small droplets (≤ 5 µm in diameter) that can evaporate into 'droplet nuclei' and as such reside in air for a longer time (WHO, 2014).

The droplets produced by sneezing are quite large in diameter, approximately ~100 µm (Han *et al.*, 2013). However, the diameter of droplet nuclei produced by coughing is distributed between 0.58 to 5.42 µm, with the majority being in sizes 0.74–2.12 µm (Yang *et al.*, 2007). Similar results have been described by Johnson *et al.*, 2011.

More and more research about COVID-19 airborne transmission has emerged. In studies by Hawks *et al.* (2021) and Port *et al.* (2022), efficient transmission of SARS-CoV-2 among hamsters via particles smaller than 5 µm in diameter was detected.

SARS-CoV-2 genes have been detected in aerosol particles that range in diameter between < 0.25 and 1 µm (Liu *et al.*, 2020) as well as 1–4 µm and > 4 µm (Chia *et al.*, 2020).

For comparison, it is known that the average volume of many bacteria ranges between ~0.4–3 µm<sup>3</sup> (Levin and Angert, 2015). The diameter of common spherical bacteria is approximately 0.2–2.0 µm, for example, *Staphylococci* are about 0.5 to 1.5 µm in diameter (Götz *et al.*, 2006).

The prevention of COVID-19, caused by SARS-CoV-2, has been one of the most topical subjects for scientists as well as general population since the pandemic began early in 2020 (WHO, 2020; Kumar *et al.* 2021). The prevention methods and infection control measures stated by the World Health Organisation (WHO, 2020) include social distancing, hand hygiene, adequate indoor ventilation systems, respiratory etiquette, avoidance of touching one's face, testing, contact tracing, quarantine, and isolation. The use of face masks is named by the WHO as one of the most significant methods of preventing COVID-19 transmission. Face masks are masks that cover the user's nose and mouth. A predecessor of the face masks we use nowadays — a gauze mask was first introduced by Johann von Mikulicz Radecki in 1897. Since then, the manufacturing, the use and the understanding of face masks has greatly evolved (Gandhi *et al.*, 2020; Matuschek *et al.*, 2020). Face masks are commonly used for protection from respiratory infections as a physical barrier against potentially infectious droplets (Jefferson *et al.*, 2009). It is known that the face masks show

their protective abilities in two main aspects — source control (protecting others from the infected wearer) and wearer protection (protecting the wearer from inhalation of infectious agents) (WHO, 2020).

In April 2020, the WHO issued recommendations for face mask use among health care workers and symptomatic, ill individuals, whereas the use of masks by healthy individuals in the community setting was not supported (WHO, 2020). In June 2020, the WHO updated the recommendations for face mask use, encouraging face mask use for the general public, in vulnerable populations, in crowded living or working conditions, and public settings (WHO, 2020). The use of face masks has been suggested or even mandatory required during peaks of disease in many countries (Yan *et al.*, 2021).

The aim of this study was to investigate the microbial, primary bacterial, contamination of used face masks to determine the efficacy of facial mask use against COVID-19 with a bacterial model. We also aimed to compare the results based on mask usage time.

## MATERIALS AND METHODS

In this study, 51 used face masks were analysed by using a bacterial model in the laboratory of Rīga Stradiņš University Department of Biology and Microbiology.

**Face masks.** In this research, two different types of face masks were included — 49 surgical masks and two cloth masks. Inclusion criteria were: surgical or cloth face masks used for at least one hour.

Exclusion criteria were: respirators and other types of face coverings; damaged face mask. The used face masks were gathered from Rīga Stradiņš University staff and students voluntarily. The usage time for each mask was inquired. After obtaining each mask, it was immediately transported to the laboratory in a sterile plastic bag. Five unused sterile face masks were used as a negative control.

**Imprinting and cultivation.** The two sides of the face masks were separately imprinted (Gund *et al.*, 2021) on sterile blood agars (TSA, Oxoid™, Basingstoke, United Kingdom) plus 6% sterile sheep blood). The inner part of the face mask (which was exposed to the skin, mouth and nose of the research participant during use) was imprinted on one sterile solid blood agar plate. The outer part (which was exposed to the external environment during use) was then imprinted on another sterile solid blood agar plate. The imprinting was carried out in aseptic conditions to avoid contamination with microorganisms in the air. The samples were then incubated for 48 hours at 37 °C.

**Bacterial growth evaluation and identification of bacteria.** The number of colony-forming units (CFU) was calculated by an automatic colony counter (Scan® 300, Interscience, France). Morphological growth evaluation of the colonies was executed to determine the variety of bacteria

found on the plates. The morphological parameters that were examined were colony size, colony shape, colour. A pure culture was obtained from each colony. The obtained pure cultures were stained using the Gram stain method and evaluated by microscopy (Nikon Eclipse E200, Japan). Identification of bacteria was performed using the automated VITEK2 method (Compact 30 bioMérieux, Marcy-l'Étoile, France).

**Fungal growth evaluation.** All of the face masks in this research were also tested for fungal growth. The inner and outer part of the face masks were imprinted on separate *Sabouraud* CAF agars (Liofilchem®, Italy). The plates were incubated in 25 °C for five days. After incubation, the number of CFU was calculated manually. Morphological growth of fungi was evaluated to determine the growth of the two basic fungal morphological forms, yeasts and hyphae. For further evaluation, the yeast colonies were coloured with methylene blue and microscopied.

**Statistical analysis.** The collected data was entered in Microsoft Excel and then statistically analysed using IBM SPSS Statistics 27. The data was skewed in most of the variable groups. The Wilcoxon test was applied to compare the insides to the outsides of the used face masks to test for significant differences in bacterial richness (species number) and counts of colony forming units, considering that both sides of these masks came from one user. The Mann–Whitney-U test was applied for comparing bacterial richness and counts of colony forming units separately on the outsides and insides of used face masks after dividing them into two groups based on the usage time — (1) masks used no more than two full hours and (2) masks used more than two hours. Statistical significance was assumed if the *p* value was less than or equal to 0.05.

## RESULTS

**Bacterial colonisation of the face masks.** Bacterial colonisation was detected on all 51 of the used face masks, both on the inside and outside (Fig. 1). Microorganisms were not found on any of the control group masks.

The majority of microorganisms found were those of normal human skin and respiratory tract microbiota, although extraneous microorganisms were also found. Overall, the most commonly found bacteria were Gram-positive bacteria — *Staphylococcus* spp. — found on 100% of the face masks (*n* = 51), followed by *Micrococcus* spp. found on 96% of the face masks (*n* = 49).

Other Gram-positive bacteria found were *Kocuria* spp., *Granulicatella* spp., *Enterococcus* spp. The most frequently found Gram negative bacteria were *Sphingomonas* spp. — found on 63% (*n* = 32) of the face masks, followed by *Moraxella* spp. (47%, *n* = 24) and *Pseudomonas* spp. (37%, *n* = 19). Other Gram-negative bacteria detected were *Rhizobium* spp., *Aeromonas* spp., *Pasteurella* spp., and *Acinetobacter* spp. (Table 1).

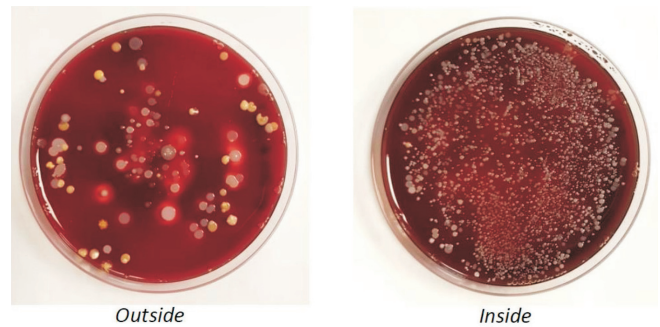


Fig. 1. Sheep blood agars after cultivation for comparison of outside and inside of one of the used face masks.

Table 1. Bacterial contamination of the used face masks

Gram stain	Identified genera	Identified species (in alphabetical order)	Amount of colonised face masks		
Gram positive	<i>Staphylococcus</i> spp.	<i>S. aureus</i>	100% ( <i>n</i> = 51)		
		<i>S. capitis</i>			
		<i>S. epidermidis</i>			
		<i>S. haemolyticus</i>			
		<i>S. hominis hominis</i>			
		<i>S. lugdunensis</i>			
		<i>S. saprophyticus</i>			
		<i>S. warneri</i>			
		<i>Micrococcus</i> spp.		<i>M. luteus</i>	96% ( <i>n</i> = 49)
		<i>Kocuria</i> spp.		<i>K. kristinae</i>	51% ( <i>n</i> = 26)
<i>K. rosea</i>					
<i>Granulicatella</i> spp.	<i>G. elegans</i>	16% ( <i>n</i> = 8)			
	<i>Enterococcus</i> spp.		<i>E. casseliflavus</i>	14% ( <i>n</i> = 7)	
Gram negative	<i>Sphingomonas</i> spp.	<i>S. paucimobilis</i>	63% ( <i>n</i> = 32)		
		<i>Moraxella</i> spp.			47% ( <i>n</i> = 24)
				<i>Pseudomonas</i> spp.	
		<i>P. oryzihabitans</i>			
		<i>P. stutzeri</i>			
		<i>Rhizobium</i> spp.		<i>R. radiobacter</i>	22% ( <i>n</i> = 11)
				<i>Aeromonas</i> spp.	
		<i>Pasteurella</i> spp.		<i>P. multocida</i>	6% ( <i>n</i> = 3)
		<i>Acinetobacter</i> spp.		<i>A. lwoffii</i>	4% ( <i>n</i> = 2)

**Bacterial species richness on the outsides and insides of the used face masks.** Tables 2–3 display bacteria, divided by genus, found on the face masks (Tables 2, 3). The median species number ( $Q_1$ – $Q_3$ ) of different bacterial colonies found on the outsides of the used face masks was 7 (5–10). The median on the insides was 4 (2–6). A Wilcoxon signed-rank test indicated that this difference in bacterial richness comparing outsides to the insides of used face masks was statistically significant,  $z = -5.328$ ,  $p < 0.001$  (Fig. 2).

**Count of CFU on the outsides and insides of the used face masks.** The median ( $Q_1$ – $Q_3$ ) count of CFU found on insides was  $6.4 (2.8–8.2) \times 10^2$ . The median count of CFU on the outsides was  $1.8 (0.8–2.8) \times 10^2$ . Count of CFU on the insides statistically significantly higher than on the outsides (Wilcoxon signed-ranks test,  $z = -5.582$ ,  $p < 0.001$ ) (Fig. 3).

Table 2. Bacteria found on the outsides of the used face masks

Bacteria found on the outsides of the face masks	Number of face masks on which the bacteria were found	% of masks with the found bacteria
<i>Staphylococcus spp.</i>	51	100.0
<i>Micrococcus spp.</i>	48	94.1
<i>Sphingomonas spp.</i>	30	58.8
<i>Kocuria spp.</i>	25	49.0
<i>Pseudomonas spp.</i>	19	37.3
<i>Moraxella spp.</i>	15	29.4
<i>Aeromonas spp.</i>	8	15.7
<i>Rhizobium spp.</i>	8	15.7
<i>Granulicatella spp.</i>	7	13.7
<i>Enterococcus spp.</i>	7	13.7
<i>Pastereulla spp.</i>	3	5.9
<i>Acinetobacter spp.</i>	1	2.0

Table 3. Bacteria found on the insides of the used face masks

Bacteria found on the insides of the face masks	Number of face masks on which the bacteria were found	% of masks with the found bacteria
<i>Staphylococcus spp.</i>	51	100.0
<i>Micrococcus spp.</i>	30	58.8
<i>Moraxella spp.</i>	15	29.4
<i>Kocuria spp.</i>	9	17.6
<i>Pseudomonas spp.</i>	6	11.8
<i>Sphingomonas spp.</i>	5	9.8
<i>Granulicatella spp.</i>	4	7.8
<i>Rhizobium spp.</i>	4	7.8
<i>Aeromonas spp.</i>	3	5.9
<i>Acinetobacter spp.</i>	1	2.0

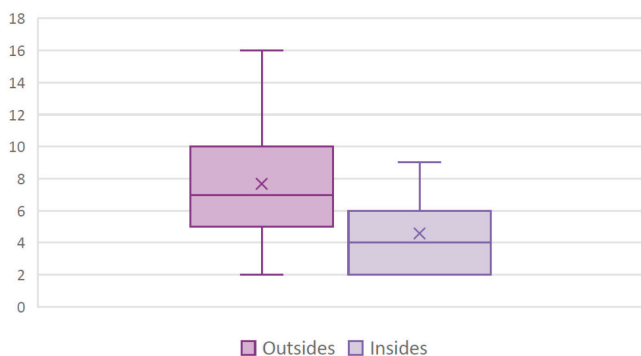


Fig. 2. Number of bacterial species on the outsides and insides of used face masks.

**Comparison of bacterial colonisation depending on the usage time.** The results were divided and compared in two groups based on the face mask usage time: (1) usage time no more than two full hours, (2) usage time three and more hours. The outsides and insides of the face masks were analysed separately. The median (Q1–Q3) CFU on the outsides of the used face masks with usage time no more than two full hours was  $1.4 (0.7–2.7) \times 10^2$ , whereas in the group of usage time three and more hours —  $2.2 (0.8 – 3.8) \times 10^2$ . The difference was statistically not significant (Mann–Whitney U = 267.500,  $p = 0.286$ ).

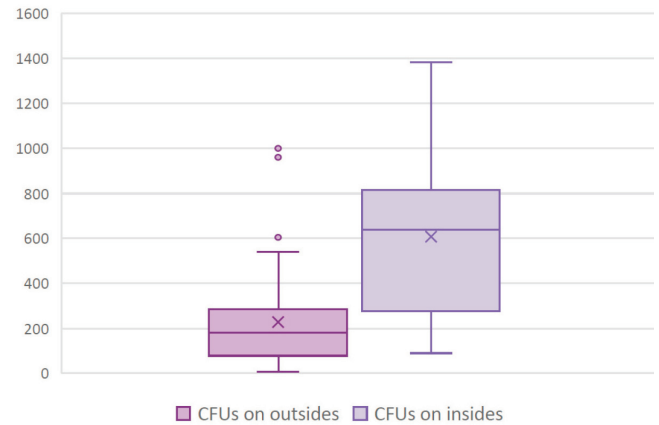


Fig. 3. CFU on the outsides and insides of used face masks.

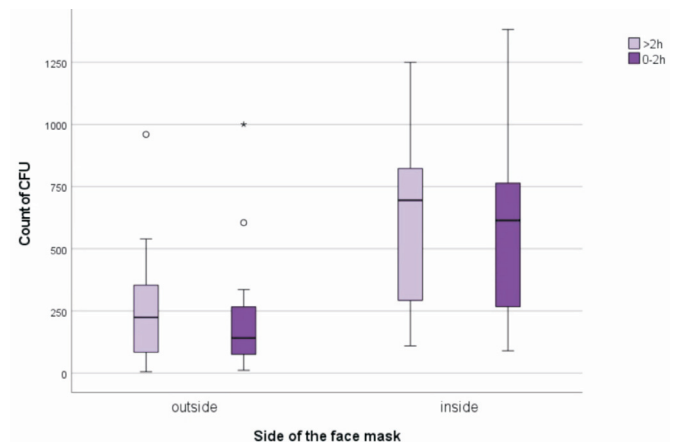


Fig. 4. CFU on the outsides and insides of used face masks comparing by the usage time.

A Mann–Whitney U test also showed that the count of CFU on the insides was not significantly greater for the masks used more than two hours (Mdn =  $7.0 (2.9–8.3) \times 10^2$ ) than for the masks used no more than two hours (Mdn =  $6.1 (2.6–7.8) \times 10^2$ ), U = 282.000,  $p = 0.428$  (Fig. 4). A Mann–Whitney U test indicated that the bacterial richness on the outsides of the face masks was significantly greater for masks used more than two hours (Mdn =  $8.0 (7.0–11.5) \times 10^2$ ) than for the masks used no more than two hours (Mdn =  $6.0 (5.0 – 8.0) \times 10^2$ ), U = 209.500,  $p = 0.029$ . The bacterial richness on the insides was not significantly greater for masks used more than two hours (Mdn =  $6.0 (2.3–6.8) \times 10^2$ ) than for the masks used no more than two hours (Mdn =  $4.0 (2.0–5.0) \times 10^2$ ), Mann–Whitney U = 249.000,  $p = 0.148$  (Fig. 5).

**Fungal colonisation.** Overall fungal colonisation was detected on 62.7% ( $n = 32$ ) of the facial masks. On the used face masks, moulds were more commonly detected. Moulds were found on the outsides of 19 masks and on the insides of 11 masks. Yeasts were found on the outsides of 11 masks and on the insides of 14 masks. On the outsides of the used face masks, fungal colonisation was found on 54.9% ( $n = 28$ ) whereas on the insides on 51.0% ( $n = 26$ ) of the face masks. A McNemar test showed no statistically significant

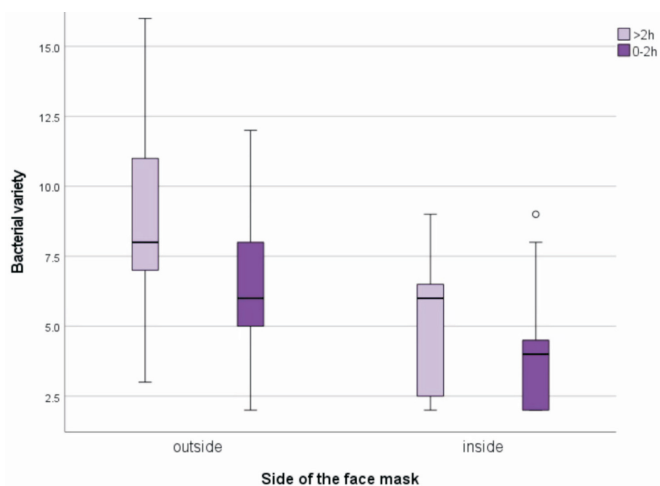


Fig. 5. Bacterial species number on the outsides and insides of used face masks compared by the usage time.

differences with fungal growth incidence, comparing the outsides to the insides ( $p = 0.754$ ). A Wilcoxon test indicated no significant difference in the count of CFU on the outsides (Mdn = 3.5 ( $Q_1 = 1.0 - Q_3 = 9.0$ )) compared to insides (Mdn = 2.0 ( $Q_1 = 1.0 - Q_3 = 4.0$ ),  $z = -1.162$ ,  $p = 0.245$ ). A Wilcoxon test showed no significant difference in the richness of fungal colonies on the outsides (Mdn = 2.0 ( $Q_1 = 1.0 - Q_3 = 2.0$ )) compared to insides (Mdn = 1.0 ( $Q_1 = 1.0 - Q_3 = 2.0$ ),  $z = -0.894$ ,  $p = 0.371$ ).

## DISCUSSION

There have been many studies investigating the protective properties of face mask use in respiratory infection transmission especially in the COVID-19 aspect. In a meta-analysis by Chu *et al.*, 2020, it was found that face mask use could “result in a large reduction in risk of infection, with stronger associations with N95 or similar respirators compared with disposable surgical masks or similar”, however, face masks alone do not have cogent and sufficient protective properties for complete prevention of infection transmission. Worby and Chang (2020) with the help of mathematical modelling showed that wearing face masks can reduce total infections and deaths from COVID-19, despite their imperfect protective effect. A systematic review and meta-analysis (Liang *et al.*, 2020) suggested that mask use provided a significant protective effect and could reduce the risk of respiratory virus infection by 80% for health-care workers and 47% for non-healthcare workers. Another study (Wang *et al.*, 2020) found that face masks were 79% effective in preventing transmission in households in Beijing, China with at least one laboratory confirmed SARS-CoV-2 infection, if they were used by all household members prior to symptom onset. Various face masks (cotton, surgical, N95) have been proved to effectively limit the transmission of respiratory droplets and aerosols containing SARS-CoV-2, but the masks could not completely block the virus transmission (Ueki *et al.* 2020). Other academic studies have also shown face mask use as a relevant and constitutive prevention method for COVID-19 (Brooks *et*

*al.*, 2020; Leung *et al.*, 2020; Zhang *et al.*, 2020; Cheng *et al.*, 2021).

The protection that the face masks can provide in retaining excreted microorganisms was well shown in the bacterial colony forming unit count difference — on the insides of the used face masks it was three times greater than for outsides of the same masks. These findings coincide with another study (Park *et al.*, 2022), where the count of CFU was significantly higher on the face-side of the face masks.

The most commonly found bacteria were Gram positive cocci — *Staphylococci*, that are about 0.5 to 1.5  $\mu\text{m}$  in diameter (Götz *et al.*, 2006), and *Micrococcus* spp., for example, *M. luteus* that were 0.5 to 3.5  $\mu\text{m}$  in diameter (Rakhashiya *et al.*, 2015). Comparing the commonly found bacteria size in diameter to the diameter of previously described respiratory particles, we can estimate that if the face masks have the ability to withhold on their surface the bacteria, they should retain the respiratory droplets and aerosols of the same size containing viruses such as COVID-19.

Other similar studies evaluating microbial contamination showed some similarities and differences of the found microbial spectrum. The most often found bacteria in this study — *Staphylococcus* spp. — were found in other studies as well in multiple occasions. In a study where face masks used by surgeons were investigated (Delanghe *et al.*, 2021) the most common bacteria were *Bacillus* spp., *Staphylococcus* spp. and *Acinetobacter* spp. Other studies reported the most isolated bacteria from face masks to be *Staphylococcus* spp. and *Pseudomonas* spp. (Liksamijarulkul *et al.*, 2014) or *E. coli* and *S. aureus* (Monalisa *et al.*, 2017).

In our study, the majority of found microorganisms on the used face masks — members of *Staphylococcus* spp., *Micrococcus* spp., *Kocuria* spp., and *Granulicatella* sp. — are those of normal human skin and respiratory tract microbiota (Aas *et al.*, 2005; Hetem *et al.*, 2017; Kandi *et al.*, 2016). These were also found mainly on the insides of the used face masks. The outsides of the masks contained more environmental microbes, such as members of *Sphingomonas* spp., *Pseudomonas* spp., *Rhizobium* spp., *Aeromonas* spp., and *Pasteurella* spp., which can cause opportunistic infections (Leys *et al.*, 2004; Lai *et al.*, 2004; Pepper and Gerba, 2009; Batra *et al.*, 2016; Hasan and Hug, 2022). Of course, it is also important to note the possibility of opportunistic infection from normal microbiome members, especially, in immunocompromised persons. The finding of common but potentially pathogenic bacteria like *S. aureus* and *S. saprophyticus* that were detected in our research has also been described in a study by Park *et al.*, 2022. In some cases, extraneous microorganisms were also found, for example, one of the face masks on the outside contained *Enterococcus columbae*, which has been previously isolated from pigeon’s intestinal tract (Dolka *et al.*, 2020).

As the insides showed mostly normal microbiota, whereas the outsides — more extraneous microorganisms, these data indicate the mask’s ability to retain exhaled microorganisms

on the insides and suggest the contamination from environment on the outsides of face masks. In cases when bacteria that is normally found in the environment was detected on either side of the face masks, we should also consider improper mask use, for example, touching the mask with unwashed hands, as a transmission possibility for these microbes. These findings coincide with the WHO recommendations of correct face mask use, emphasising the importance of appropriate face mask use, storage and disposal for their maximum effectiveness and reduced transmission risk (WHO, 2020). It is important to note a limitation in this study that could have affected the results — the study did not record whether the mask wearer was aware of the recommendations for correct use of the mask and whether they had followed it.

When comparing the groups of face masks used no more than two hours to masks used more than two hours, we expected that both the count of CFU and the bacterial variety would increase on both sides of the face masks. Even though a tendency for increased count of CFU over usage time was observed, there was no statistically significant difference of CFU found on the insides and outsides of the used face masks comparing the groups of masks used no more than two hours versus those used more than two hours. This result could be explained by the small number of masks and the usage time difference distribution we chose for comparison. For the continuation of the study, we should evaluate masks with more different usage time.

The bacterial variety on the outsides was significantly higher comparing face masks used more than two hours versus those used no more than two hours, whereas on the insides a statistically significant difference was not proven. Once more, these results demonstrate the ability of the face masks to retain microorganisms that people excrete when they speak, breathe, cough — the microbiome of the mask user's skin, oral cavity and upper respiratory tract is inalterable. The increased bacterial species richness on the outsides with prolonged usage time furthermore indicates the risks of how inappropriate face mask use could increase the risk of potentially pathogenic microorganism transmission. These findings emphasise the importance of appropriate face mask use and disposal to ensure their effectiveness and reduce increased transmission of pathogenic and potentially pathogenic bacteria as well as SARS-CoV-2.

In this study we also tried to analyse the difference between simple surgical masks versus cloth masks. Unfortunately, the group with cloth masks was significantly smaller, due to the fact that those who wore it did not want to donate it to research. We included cloth masks in the study, however due to this small sample size of cloth masks, we decided to not divide the cloth masks into a separate group when analysing the results. However, the few samples showed that the microbial colonisation on the cloth masks was quite remarkable. An explanation for this might be that cloth masks were more often used inappropriately, with taking them off and putting back on more than once as well as washing them rarely. As a limitation in this study, we should also

note that we could not follow whether or not the recommendations for correct face mask use were respected by the research subjects.

With the bacterial model we can clearly demonstrate the face mask ability to reduce the transmission of excreted microorganisms. A limitation of this study was the difference in bacterial sizes and the respiratory droplet nuclei, as their diameters can vary. There are newly published and still ongoing studies about the sizes of aerosol particles in which SARS-CoV-2 can be found. A growing amount of data proving COVID-19 transmission with aerosol particles is available, showing the need for better comparison of bacteria and the respiratory droplets to fully apply results about bacterial contamination to SARS-CoV-2. By comparing the data obtained in our study with previous studies, we concluded that the effectiveness of the masks is significant, but further research is needed on the shortcomings of the masks.

## CONCLUSIONS

The most commonly found microorganisms on used face masks were members of normal microbiota — *Staphylococcus* spp., and *Micrococcus* spp. Extrinsic microorganisms were also found, more commonly on the outsides. More CFU were detected on the insides of the face masks than on the outsides, reflecting the ability of facial masks to detain excreted microorganisms. The outsides of face masks contained a wider variety of bacterial species, furthermore, the bacterial species number increased over usage time on the outsides on the face masks but not on the insides. These results emphasise the importance of correct facial mask usage showing that inappropriate face mask use increases the risk of potentially pathogenic microorganism transmission.

## REFERENCES

- Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. *J. Clin. Microbiol.*, **43** (11), 5721–5732.
- Alsved, M., Matamis, A., Bohlin, R., Richter, M., Bengtsson, P.-E., Fraenkel, C.-J., Medstrand, P., Löndahl, J. (2020). Exhaled respiratory particles during singing and talking. *Aerosol Sci. Technol.*, **54** (11), 1245–1248.
- Asadi, S., Wexler, A. S., Cappa, C. D., Barreda, S., Bouvier, N. M., Ristenpart, W. D. (2019). Aerosol emission and superemission during human speech increase with voice loudness. *Sci. Rep.*, **9** (1), 2348.
- Batra, P., Mathur, P., Misra, M. C. (2016). *Aeromonas* spp.: An emerging nosocomial pathogen. *J. Labor. Phys.*, **8** (1), 1–4.
- Brooks, J. T., Butler, J. C., Redfield, R. R. (2020). Universal masking to prevent SARS-CoV-2 transmission — The time is now. *JAMA*. **324** (7), 635–637.
- Cascella, M., Rajnik, M., Aleem, A., Dulebohn, S. C., Di Napoli, R. (2022). Features, evaluation, and treatment of coronavirus (COVID-19). In: *StatPearls*. StatPearls Publishing.
- Cheng, Y., Ma, N., Witt, C., Rapp, S., Wild, P. S., Andreae, M. O., Pöschl U., Su, H. (2021). Face masks effectively limit the probability of SARS-CoV-2 transmission. *Science*, **372** (6549), 1439–1443.

- Chia, P. Y., Coleman, K. K., Tan, Y. K., Ong, S., Gum, M., Lau, S. K., Lim, X. F., Lim, A. S., Sutjipto, S., Lee, P. H., *et al.* (2020). Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nature Commun.*, **11** (1), 2800.
- Chu, D. K., Akl, E. A., Duda, S., Solo, K., Yaacoub, S., Schünemann, H. J., on behalf of the COVID-19 Systematic Urgent Review Group Effort (SURGE) study authors (2020). Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: A systematic review and meta-analysis. *The Lancet*, **395** (10242), 1973–1987.
- Corman, V. M., Muth, D., Niemeyer, D., Drosten, C. (2018). Hosts and sources of endemic human coronaviruses. *Adv. Virus Res.*, **100**, 163–188.
- Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. (2020). The species *Severe acute respiratory syndrome-related coronavirus*: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.*, **5**, 536–544 (2020).
- Cui, J., Li, F. Shi, Z. L. (2019). Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.*, **17**, 181–192.
- De Groot, R. J., Baker, S. C., Baric, R. S., Brown, C. S., Drosten, C., Enjuanes, L., Fouchier R. A. M., Galiano M., Gorbalenya A.E., Memish Z. A., *et al.* (2013). Middle East respiratory syndrome coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *J. Virol.*, **87** (14), 7790–7792.
- Delanghe, L., Cauwenberghs, E., Spacova, I., De Boeck, I., Van Beeck, W., Pepermans, K., Claes, I., Vandenheuveld, D., Verhoeven, V., Lebeer, S. (2021). Cotton and surgical face masks in community settings: Bacterial contamination and face mask hygiene. *Frontiers Med.*, **8**. <https://www.frontiersin.org/articles/10.3389/fmed.2021.732047/full>.
- Dolka, B., Czopowicz, M., Chrobak-Chmiel, D., Ledwoń A., Szeleszczuk, P. (2020). Prevalence, antibiotic susceptibility and virulence factors of *Enterococcus* species in racing pigeons (*Columba livia f. domestica*). *BMC Vet. Res.*, **16**, 7.
- Echternach, M., Gantner, S., Peters, G., Westphalen, C., Benthous, T., Jakubaß, B., Kuranova, L., Döllinger, M., Kniesburges, S. (2020). Impulse dispersion of aerosols during singing and speaking: A potential COVID-19 transmission pathway. *Amer. J. Respir. Crit. Care Med.*, **202** (11), 1584–1587.
- Edwards, D. A., Ausiello, D., Salzman, J., Devlin, T., Langer, R., Beddingfield, B. J., Fears, A. C., Doyle-Meyers, L. A., Redmann, R. K., Killeen, S. Z., Maness, N. J., Roy, C. J. (2021). Exhaled aerosol increases with COVID-19 infection, age, and obesity. *Proc. Natl. Acad. Sci U S A*, **118** (8), 2021830118.
- Gandhi, J. A., Churiwala, J. J., Shinde, P. H., Chaudhari S. N. (2020). Surgical mask—the saviour: From its invention to the COVID-19 era. *Indian J. Surg.*, **82**, 278–279.
- Götz, F., Bannerman, T., Schleifer, K. H. (2006). The genera *Staphylococcus* and *Micrococcus*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., Stackebrandt, E. (eds.). *The Prokaryotes*. Springer, New York, NY.
- Gund, M., Isack, J., Hannig, M., Thieme-Ruffing, S., Gärtner, B., Boros, G., Rupf, S. (2021). Contamination of surgical mask during aerosol-producing dental treatments. *Clin. Oral Invest.*, **25**, 3173–3180.
- Han, Z. Y., Weng, W. G., Huang, Q. Y. (2013). Characterizations of particle size distribution of the droplets exhaled by sneeze. *J. Royal Soc., Interface*, **10** (88).
- Hasan, J., Hug, M. (2022). *Pasteurella multocida*. In: *StatPearls*. StatPearls Publishing.
- Hawks, S. A., Prussin, A. J., II, Kuchinsky, S. C., Pan, J., Marr, L. C., Duggal, N. K. (2021). Infectious SARS-CoV-2 is emitted in aerosol particles. *mBio*, **12** (5).
- Hetem, D. J., Rooijackers, S. H. M., Ekkelenkamp, M. B. (2017). Staphylococci and micrococci. In: Cohen, J., Powderly, W. G., Opal, S. M. *Infectious Diseases* (4<sup>th</sup> edn.), Elsevier, pp. 1509–1522.
- Holmes, E. C., Rambaut, A. (2004). Viral evolution and the emergence of SARS coronavirus. *Phil. Trans. R. Soc. Lond. B* **359**, 1059–1065.
- Jefferson, T., Del Mar, C., Dooley, L., Ferroni, E., Al-Ansary, L. A., Bawazeer, G. A., van Driel, M. L., Foxlee, R., Rivetti, A. (2009). Physical interventions to interrupt or reduce the spread of respiratory viruses: systematic review. *BMJ*, **339**, b3675.
- Johnson, G. R., Morawska, L., Ristovski, Z. D., Hargreaves, M., Mengersen, K., Chao, C. Y. H., Wan, M. P., Li, Y., Xie, X., Katoshevski, D. S. Corbett, S. (2011). Modality of human expired aerosol size distributions. *J. Aerosol Sci.*, **42** (12), 839–851.
- Kandi, V., Palange, P., Vaish, R., Bhatti, A. B., Kale V., Kandi M., R., Bhoomagiri M. R. (2016). Emerging bacterial infection: Identification and clinical significance of *Kocuria* species. *Cureus*, **8** (8), e731.
- Kim, J. M., Chung, Y. S., Jo, H. J., Lee, N. J., Kim, M. S., Woo, S. H., Park, S., Kim, J. W., Kim, H. N., Han, M. G. (2020). Identification of Coronavirus isolated from a patient in Korea with COVID-19. *Osong Publ. Health Res. Perspect.*, **11** (1), 3–7.
- Kumar, V., Alshazly, H., Idris, S. A., Bourouis, S. (2021). Impact of COVID-19 on society, environment, economy, and education. *Sustainability*, **13** (24), 13642.
- Lai, C.-C., Teng, L.-J., Hsueh, P.-R., Yuan, A., Tsai, K.-C., Tang, J.-L., Tien, H.-F. (2004). Clinical and microbiological characteristics of *Rhizobium radiobacter* infections. *Clin. Infect. Dis.*, **38** (1), 149–153.
- Leung, N., Chu, D., Shiu, E., Chan, K. H., McDevitt, J. J., Hau, B., Yen, H. L., Li, Y., Ip, D., Peiris, J., Seto, W. H., Leung, G. M., Milton, D. K., Cowling, B. J. (2020). Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nature Med.*, **26** (5), 676–680.
- Levin P. A., Angert E. R. (2015). Small but mighty: Cell size and bacteria. *Cold Spring Harb. Perspect. Biol.*, **7** (7), a019216.
- Leys, N. M. E. J., Ryngaert, A., Bastiaens, L., Verstraete, W., Top, E. M., Springael, D. (2004). Occurrence and phylogenetic diversity of *Sphingomonas* strains in soils contaminated with polycyclic aromatic hydrocarbons. *Appl. Environ. Microbiol.*, **70** (4), 1944–1955.
- Liang, M., Gao, L., Cheng, C., Zhou, Q., Uy, J. P., Heiner, K., Sun, C. (2020). Efficacy of face mask in preventing respiratory virus transmission: A systematic review and meta-analysis. *Travel Med. Infect. Dis.*, **36**, 101751.
- Liksamijarulkul, P., Aiempradit, N., Vatanasomboon, P. (2014) Microbial contamination on used surgical mask among hospital personnel and microbiological air quality in their working ward: A hospital in Bangkok. *Oman Med. J.*, **29** (5), 346–350.
- Li, F. (2016). Structure, function, and evolution of coronavirus spike proteins. *Annu. Rev. Virol.*, **3** (1), 237–261.
- Liu, Y., Ning, Z., Chen, Y., Guo, M., Liu, Y., Gali, N. K., Sun, L., Duan, Y., Cai, J., Westerdahl, D., *et al.* (2020). Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*, **582**, 557–560.
- Masters, P. S., Perlman, S. (2013). Coronaviridae. In: Knipe, D. M., Howley, P. M. *Fields Virology*. 6<sup>th</sup> edn. Lippincott Williams & Wilkins, pp. 825–858.
- Matuschek, C., Moll, F., Fangerau, H., Fischer, J. C., Zänker, K., van Griensven, M., Schneider, M., Kindgen-Milles, D., Knoefel, W. T., Lichtenberg, A., *et al.* (2020). The history and value of face masks. *Eur. J. Med. Res.*, **25**, 23.
- Monalisa, Aruna, C. N. Bhat, P. K., Manjunath, K., Hemavathy, E., Varsha, D. (2017). Microbial contamination of the mouth masks used by postgraduate students in a private dental institution: An *in-vitro* study. *IOSR J. Dental Med. Sci.*, **16** (5), 61–67.
- Park, A. M., Khadka, S., Sato, F., Omura S., Fujita M., Hashiwaki K., Tsunoda I. (2022). Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Sci. Rep.*, **12**, 11361.
- Park, W. B., Kwon, N. J., Choi, S. J., Kang, C. K., Choe, P. G., Kim, J. Y., Yun, J., Lee, G. W., Seong, M. W., Kim, N. J., Seo, J. S., Oh, M. D. (2020).

- Virus isolation from the first patient with SARS-CoV-2 in Korea. *J. Korean Med. Sci.*, **35** (7), e84.
- Pepper, I. A., Gerba, C. P. (2009). Cultural methods. In: Maier, R. M., Pepper, I. L., Gerba, C. P. *Environmental Microbiology*. 2<sup>nd</sup> edn. Academic Press, pp. 173–189.
- Port, J. R., Yinda, C. K., Avanzato, V. A., Schulz, J. E., Holbrook, M. G., van Doremalen, N., Shaia, C., Fischer, R. J., Munster, V. J. (2022). Increased small particle aerosol transmission of B.1.1.7 compared with SARS-CoV-2 lineage A *in vivo*. *Nat. Microbiol.*, **7**, 213–223.
- ProMED-mail. <https://promedmail.org/promed-post/?id=6864153> (accessed 30.09.2022).
- Rakhashiya, P. M., Patel, P. P., Thaker, V. S. (2015). Whole genome sequences and annotation of *Micrococcus luteus* SUBG006, a novel phytopathogen of mango. *Genomics Data*, **6**, 10–11.
- Wang, Y., Tian, H., Zhang, L., Zhang, M., Guo, D., Wu, W., Zhang, X., Kan, G. L., Jia, L., Huo, D., *et al.* (2020). Reduction of secondary transmission of SARS-CoV-2 in households by face mask use, disinfection and social distancing: A cohort study in Beijing, China. *BMJ Global Health*, **5** (5), e002794.
- Ueki, H., Furusawa, Y., Iwatsuki-Horimoto, K., Imai, M., Kabata, H., Nishimura, H., Kawaoka, Y. (2020). Effectiveness of face masks in preventing airborne transmission of SARS-CoV-2. *mSphere*, **5** (5), e00637-20.
- Worby, C. J., Chang, H. H. (2020). Face mask use in the general population and optimal resource allocation during the COVID-19 pandemic. *Nat. Commun.*, **11**, 4049.
- World Health Organization. (2014). Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. Geneva: World Health Organization. <https://www.who.int/publications/i/item/infection-prevention-and-control-of-epidemic-and-pandemic-prone-acute-respiratory-infections-in-health-care> (accessed 12.01.2024).
- World Health Organization. (2020). Advice on the use of masks in the context of COVID-19: interim guidance, 6 April 2020. World Health Organization. <https://iris.who.int/handle/10665/331693> (accessed 12.01.2024).
- World Health Organization. (2020). Advice on the use of masks in the context of COVID-19: interim guidance, 5 June 2020. World Health Organization. <https://iris.who.int/handle/10665/332293> (accessed 12.01.2024).
- World Health Organization. (2020). Mask use in the context of COVID-19: interim guidance, 1 December 2020. World Health Organization. [https://www.who.int/publications/i/item/advice-on-the-use-of-masks-in-the-community-during-home-care-and-in-healthcare-settings-in-the-context-of-the-novel-coronavirus-\(2019-ncov\)-outbreak](https://www.who.int/publications/i/item/advice-on-the-use-of-masks-in-the-community-during-home-care-and-in-healthcare-settings-in-the-context-of-the-novel-coronavirus-(2019-ncov)-outbreak) (accessed 12.01.2024).
- Yan, Y., Bayham, J., Richter, A., Fenichel, E. P. (2021). Risk compensation and face mask mandates during the COVID-19 pandemic. *Sci. Rep.*, **11**, 3174.
- Yang, S., Lee, G. W. M., Chen, C.-M., Wu, C.-C., Yu, K.-P. (2007). The size and concentration of droplets generated by coughing in human subjects. *J. Aerosol Med.*, **20** (4), 484–494.
- Zhang, R., Li, Y., Zhang, A. L., Wang, Y., Molina, M. J. (2020). Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proc. Natl. Acad. Sci.*, **117** (26) 14857–14863.
- Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., *et al.* (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, **579**, 270–273.
- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., *et al.* (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New Engl. J. Med.*, **382** (8), 727–733.

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## SEJAS MASKU LIETOŠANAS EFEKTIVITĀTE COVID-19 TRANSMISIJĀ, IZMANTOJOT BAKTERIĀLU MODELI

SARS-CoV-2 izraisītās Covid-19 slimības profilakse ietver infekciozo respiratoro pilienu un aerosolu pārneses samazināšanu ar dažādām metodēm, tostarp sejas masku lietošanu. Par sejas masku iedarbīgumu publicēti dažādi pētījumi – tās atzītas par lētu, taču lietderīgu profilakses metodi. Šī pētījuma mērķis bija izpētīt lietoto sejas masku mikrobiālo piesārņojumu un salīdzināt rezultātus pēc masku lietošanas laika, lai noteiktu sejas masku lietošanas efektivitāti Covid-19 infekcijas profilaksei. Tika izpētīta 51 lietota sejas maska, ar nospiedumu metodi, veicot uzņēmumus uz atbilstošiem kultivēšanas agarjiem un nosakot koloniju veidojošo vienību skaitu. Lielākā daļa atrasto mikroorganismu bija normālas cilvēka ādas un elpceļu mikrobiotas pārstāvji. Lietoto sejas masku ārpusē tika konstatēta lielāka baktēriju sugu dažādība, turklāt tika secināts, ka to palielināja ilgāks lietošanas laiks. Secinājām, ka sejas maskas lietošana ir efektīva gan izdalīto mikroorganismu aizturēšanā, gan valkātāja pasargāšanā no gaisā esošiem mikrobiem, tāpēc tā būtu jāiekļauj elpceļu slimību profilakses stratēģijās. Tomēr mūsu rezultāti uzsver arī pareizas sejas maskas lietošanas nozīmi, uzrādot masku piesārņojumu ar dažādiem mikroorganismiem.