

STAPHYLOCOCCUS SPECIES ISOLATED AT DIFFERENT TIME USAGE OF FACE MASKS AMONG UNDERGRADUATE PRECLINICAL MEDICAL STUDENTS

Loo Yong Kit¹, Wan Nur Aida Binti Wan Azman¹, Tee Wei Zhong¹, Siti Aisyah Binti Che Bahaman¹, Husna Johanna Iman¹, Lee Le¹, Lim Eileen¹, Sydney Aninthethaai¹, Gunawardena Chethila Wathsara¹, Godella Pathirage Kirani Prathiba¹, Parkkiyarajah Kisaniya¹, Hetti Arachchige Vihara Thisuli Dias¹, Yashriah Bibi Bundhoo¹, Chin Jin Han¹, Leslie Than Thian Lung², Shalini Vellasamy^{1*}.

¹ Faculty of Medicine, MAHSA University, Bandar Saujana Putra, Selangor, Malaysia

² Department of Medical Microbiology and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor

Corresponding Author(s): shalini.v@mahsa.edu.my

Published: 24th December 2025

Abstract

Background: Prolonged usage of face masks without proper hygiene could lead to disruption of skin and nasal microbiome or respiratory infections. Hence, we aim to evaluate the risk of bacterial contamination on usage of face masks between four hours and seven hours.

Methodology: Thirty (n=30) randomly selected preclinical undergraduate medical students were randomly selected to wear face masks for four hours and seven hours on different days. There was no restriction in their verbal interaction and physical activities during their mask-wearing period. After use, the masks were swabbed and cultured on Mannitol Salt Agar (MSA). Colony-forming units (CFU) were counted, followed by gram staining and coagulase test. *Staphylococcus aureus* was identified to assess contamination levels.

Results: The overall bacterial count on the surface of the face masks increased with extended wearing time. In terms of *Staphylococcus aureus*, a significant elevation of 83.33% was found between four hours and seven hours ($p < 0.05$).

Conclusion: This study suggested the longer the duration of mask usage, the higher the bacterial load observed, indicating a proportionate increase in contamination risk.

Keywords: Bacterial contamination, Usage duration, Face masks, *Staphylococcus aureus*, Preclinical medical students

1. Introduction

The era of the coronavirus disease 2019 (COVID-19) has led to the mandatory use of face masks in public settings especially in healthcare settings. The public use of face masks has been shown to be effective in limiting the transmission of COVID-19 (1,2). Moreover, individuals wearing face masks are reported to be protected from various infections (3,4). However, for a face mask to be effective, it has to be used correctly with proper storage, hand hygiene, and proper disposal of the used mask as published by the World Health Organization (WHO) (5).

Under suitable conditions, most of the bacteria and fungi have the capability to adhere, survive and multiply on the surface of various objects and materials. Face masks being a source of bacterial or viral contamination in community and clinical settings has been shown in previously conducted studies (7,9,10). Delanghe *et al.* found out that the microbiome on the surgical masks after four hours of usage mainly consisted of *Staphylococcus* spp., specifically *S. epidermidis* and *S. aureus* which are normal flora on our skin (9). Even though as skin flora, they are still capable of causing certain skin infections such as acne vulgaris and atopic dermatitis (11). Prolonged use of face masks creates a humid and occlusive environment, combined with friction and heat, which disrupts the cutaneous barrier and promotes microenvironmental changes favorable to pathogen overgrowth.

A study by Kaur T *et al.* showed that prolonged usage of mask can bring about negative cutaneous impact to the wearer such as maculopapular rash, mask-induced acne and contact dermatitis (6,19). Other than dermatological aspect of side effects, there are also reports on breathing difficulties and headache after wearing face masks for an extensive period of time (7,18). Moreover, it is also suggested that an individual may risk acquiring respiratory infections through extended use or reuse of face masks due to pathogens adherence on the used mask layer (7,8). Several literatures explained that these symptoms were mostly due to the hot, moist air trapped beneath the mask, providing an optimal environment for bacterial growth (6,7). These alterations not only exacerbate inflammatory dermatoses but also increase the risk of colonization by methicillin-resistant *Staphylococcus aureus* (MRSA), a pathogen strongly associated with hospital-acquired infections (HAIs) (23,24).

In 2020, WHO advised that the maximum time length for mask usage should be four hours as the wearer's tolerance start to deteriorate after the duration (5). The issue of wearing face masks for an extensive period of time should be addressed to the public in effort to raise their awareness. Although there are plenty of studies revolving around the effects of prolonged mask usage, there is insufficient study to come up with a definite duration of time period whereby face masks can be worn with minimal risk of self-contamination (7). Radonovich *et al.* reported that the average tolerance time of a person wearing a surgical mask is 7.7 hours (12). A multicenter cross-sectional study in China reported that almost 46% of both healthcare workers and the general population experienced skin barrier damage from mask use. Longer daily wear time was significantly associated with increased risk of respiratory diseases (25). In contrast, as mentioned earlier, WHO suggested the use of face mask of up to four hours as going beyond that duration would cause discomfort to the wearer, and hence should be avoided. While, many other of the studies did not report on the bacterial load at particular timings and there is inadequate analysis done on the bacteria load on face masks at different time periods. Therefore, more studies should be done on bacterial loads at different point of time to reinforce the recommendations on the duration that face masks can be worn with minimal risk of self-contamination that may potentially lead to respiratory infections or harmful for immunocompromised patients.

The bacterial load on the surface of the face masks at different time periods was mainly focused in this study. This study was aimed to identify the presence of *Staphylococcus aureus* that are found on the surface of the face masks by culturing the microbes isolated from the face side of the masks. *Staphylococcus* spp. became the ideal subject of interest in this study as studies showed that this species of bacterium was mostly cultured from both surfaces of the used masks (9). *Staphylococcus aureus* was utilized as a marker to evaluate the bacterial load for different duration of mask usage.

Preclinical undergraduate medical students were the pool of selection for the samples in this study. Firstly, it is one of our objectives to enhance the understanding of preclinical undergraduate medical students on the risks of prolonged usage of face masks, and secondly, it is to encourage the preclinical undergraduate medical students to practice proper mask hygiene, so they could ensure patients' safety as

well as their own when they were to attend to patients in their future years as a doctor. It is also important to acknowledge that the utilization of face masks and adherence to hygiene practices may also represent significant contributors to nosocomial infections.

2. Methodology

2.1 Study Design

A quantitative descriptive study was conducted to investigate bacterial contamination of face masks among preclinical undergraduate medical students. Thirty preclinical undergraduate medical students from Year 1 and Year 2, with age ranging from 19-26 were randomly selected as participants. Healthy participants were given new, disposable surgical face masks (MEDICOS, Malaysia) and were asked to wear it for a period of four hours and seven hours during their usual academic schedule on two separate days. There was no restriction in their verbal interaction and physical activities during their mask-wearing period. Participation in this study was voluntary. A consent form was distributed to participants to obtain their consent to be part of this study. In total, 30 face masks were used as the experimental sample size for both the stipulated time durations.

2.2 Sample Collection

On day one of the first week, face masks were provided to 15 medical students from Year 1 and 15 medical students from Year 2 to be worn for four hours. The face masks were then collected from the students and placed into sterile, individual resealable bags. Sterile gloves were used for all mask handling, and no area of the mask was touched or exposed to non-sterile surfaces. All of the collected masks were brought to the lab within an hour and kept in a cooler box at 4°C. The same procedures were repeated on the following day with the duration of face masks to be worn being seven hours.

2.3 Microbial Culture

In this experiment, Mannitol Salt Agar (MSA) agar plates were utilized to culture the microbes present on the face masks. Isolation of *Staphylococcus* spp present on the face masks were done by swabbing the face-side of the face masks using sterile cotton swabs and spreading it directly onto MSA agar plate. The plates were incubated at 37°C under aerobic condition for 48 h in an incubator.

2.4 Colony Count

To estimate the colony-forming unit (CFU) on each

agar plates, the colonies formed on the agar plates were manually counted. The concentration of the microbes grown on the culture plates were enumerated.

2.5 Gram Staining

Gram staining was performed with the colonies found on the MSA plates to differentiate the bacterial isolates. Firstly, a single colony was placed on a clean glass slide, heat-fixed, and then followed by the standard Gram staining procedure. The slide was first stained with crystal violet solution (0.1%) for 1 minute and then washed, followed by iodine (0.1%) for 1 minute and washed and then decolorized with of ethanol for 30 seconds. Lastly, safranin (0.5%) was poured onto the slides for counterstaining for 1 minute and washed. The slides were then observed under a light microscope at 1000x magnification using oil immersion for bacterial identification (14).

2.6 Coagulase test

The coagulase test was used to determine whether *Staphylococcus aureus* produced the coagulase enzyme, which allowed it to be distinguished from other *Staphylococcus* species. Using the slide method, a tiny quantity of a fresh bacterial colony was emulsified in a drop of rabbit plasma on a sterile glass slide and gently mixed. A positive result, verifying the presence of *S. aureus*, was indicated by the formation of visible clumping or agglutination within 10-30 seconds (15).

2.7 Statistical Analysis

A paired-sample t-test was conducted using SPSS 21.0 for Windows for statistical analysis of the data that were obtained. It was done to ascertain if there was a significant difference between the bacterial loads at two points of time. The significance level was set at $p < 0.05$.

3. Results

This study shows that under normal settings, face masks may accumulate significant bacterial loads, including potential pathogenic species. The bacterial load of 30 surgical face masks worn by 30 preclinical undergraduate medical students for four hours and seven hours were determined. The four-hour cut-off point in this study was decided based on WHO, as they suggested the average safe time range a face mask can be worn is up to four hours (5). There are studies which have had their participants wearing the face mask for up to eight hours. The outcome of these studies showed that the participants had significant decrease in their well-being, such as having headaches, difficulty in

breathing and discomfort (7,12). Therefore, considering the participants' well-being, we chose a safe period of seven hours.

Representing the total number of colonies after four hours of wearing the surgical face masks, statistically, they contained a mean of 32.667 (SD = 20.373) and for the duration of seven hours, the surgical face masks contained a mean of 42 (SD = 24.868) as shown

in Fig.1. A paired t-test was done to evaluate the difference in total number of colonies after four hours and seven hours. The results obtained showed significant difference ($P = 0.038$, $P < 0.05$) as seen in Fig.1 with the mean total number of colonies after seven hours (42 ± 10.152) [mean \pm standard error of the mean (SEM)] being higher compared to that of after four hours (32.667 ± 8.317) [mean \pm SEM] shown in Fig.1.

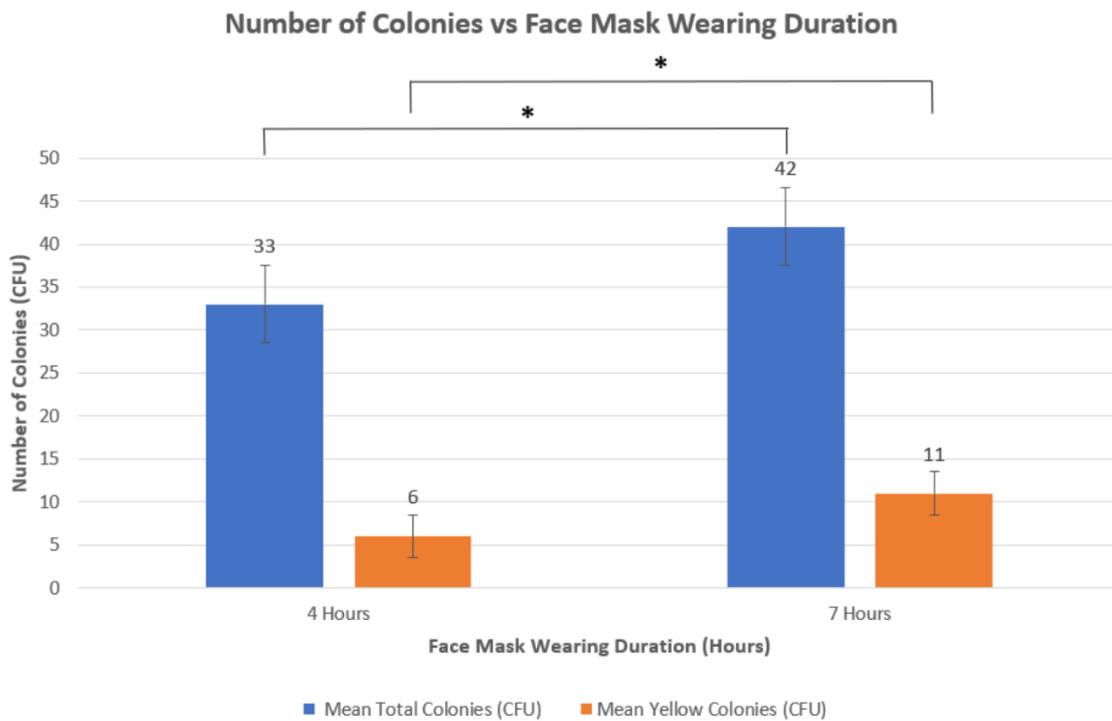


Fig 1: The mean and standard error of the mean (SEM) of number of colonies against face mask wearing time. The longer the wearing time, the higher the colony count., (*) significant difference: $p = 0.05$

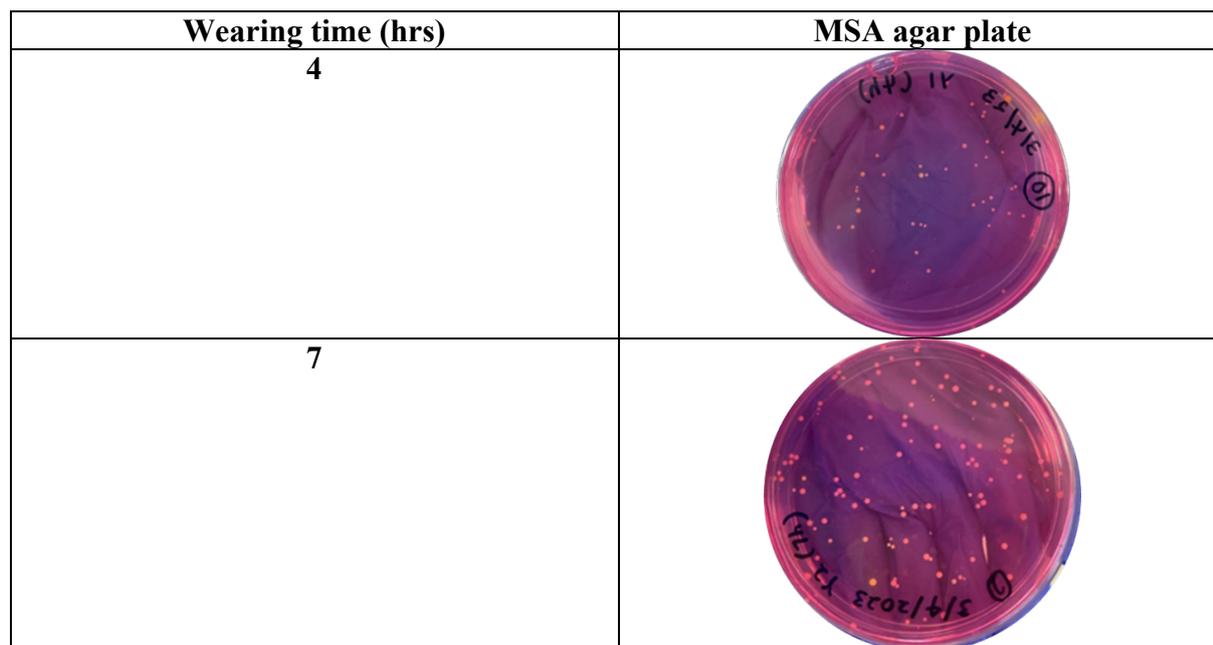
The bacterial count on the face-side of the surgical face masks increased by 27.27% with prolonged wearing time when compared between four hours and seven hours. Particularly concerning are opportunistic pathogens like *Corynebacterium*, *Staphylococcus* which can cause infections when host immunity is weakened even though they are a natural element of the human microbiota (20). These opportunistic organisms can survive and possibly grow in the presence of favorable conditions such as humidity and nutrition availability on mask surfaces, raising the risk of skin irritation, respiratory tract infections, and in extreme situations, systemic disease (21).

Additionally, prolonged mask usage may unintentionally act as a transient reservoir for resistant strains of these infections because some of them have acquired or intrinsic antimicrobial resistance pathways (22).

As for yellow colony count, a significant elevation of 83.33% was observed between both the duration of wearing time as seen in Fig. 2. The mean and standard error of the mean (SEM) were shown in the bar chart as shown in table 1.

Table I: Average number of colonies on face masks after a duration of four and seven hours, (*) significant difference: $P < 0.05$

Categories	Duration	n	Mean (CFU)	SD (CFU)	SEM (CFU)	p value
Total colony	4 h	15	32.667	20.373	8.317	0.038*
	7 h	15	42.000	24.868	10.152	
Yellow colony	4 h	15	6.167	5.636	2.301	0.034*
	7 h	15	11.000	6.782	2.769	

**Figure 2: The number of yellow colonies obtained on MSA agar plate at 4 and 7 hrs.**

Through this study, it was demonstrated that there were higher loads of bacteria with prolonged use of face masks through the comparison between two time points. In Fig 1, a significant trend of the bacterial load at four hours and seven hours was exhibited. The colony count of face masks after seven hours was higher.

Nonetheless, several other studies concurred with the findings in this study. A similar study conducted by Li *et al.* was based on how surgical masks could be the source of bacterial contamination during lengthy operative procedures by health care professionals (10). The results indicated that the bacterial count on the surface of the surgical masks worn by the health care professionals elevated significantly with extending operating time (8). Parallely, another study also indicated a higher bacterial load on face masks after wearing for four hours compared to the initial bacterial load on new, not-worn face masks (9). However, a study done by Park A-M *et al.* stated otherwise. They discovered that the bacterial colony

counts did not relate to the increase in duration of mask usage. They further concluded that it was possibly due to the used masks not being worn and were left untouched for a period of time, and that caused the moisture index of the face masks to be disrupted (8). Moreover, it was reported that extended mask use may provide resistant pathogenic strains with a temporary reservoir, enabling them to survive in close contact with the wearer's respiratory and dermal surfaces. Such conditions may increase the risk of cross-contamination and potential transmission of antimicrobial-resistant (AMR) bacteria, thereby contributing to the broader public health challenge of AMR (22).

Gram staining was conducted by considering the colonies grown on the MSA agar to identify the bacterial species. Both yellow and non-yellow colonies appeared as Gram-positive cocci, arranging in clusters as shown in Fig. 3. Similarly, in the study done by Park *et al.*, majority of the small yellow and non-yellow colonies were found to be Gram-positive cocci in clusters (8). An investigation of microbial growth on face masks conducted by Amir *et al.* also identified the

bacteria present on face masks are coccus in shape (13,17). Further coagulase test was subsequently performed on the isolated yellow and non-yellow colonies cultured on nutrient agar to differentiate coagulase-positive *Staphylococcus aureus* from coagulase-negative staphylococci. The yellow colonies produced coagulase-positive results, as visible clumping was observed, suggesting a high likelihood that these colonies were *S. aureus*. In contrast, the non-yellow colonies yielded coagulase-negative results, with no clumping observed (data not shown). The detection of *S. aureus* on used face masks is noteworthy, as this organism is a common opportunistic pathogen capable of causing a spectrum of infections ranging from superficial skin conditions to severe systemic diseases (20,21). Of particular concern is the potential presence of methicillin-resistant *S. aureus* (MRSA), which contributes

significantly to the global antimicrobial resistance crisis. The persistence of *S. aureus* on mask surfaces during prolonged use underscores the potential risk of transmission and highlights the importance of proper mask hygiene and timely replacement to reduce the spread of resistant pathogens (22). Prolonged usage of face masks without proper replacement greatly increases bacterial burden, including opportunistic and potentially resistant infections like *Staphylococcus aureus* (5). In clinical and community settings, inadequate mask hygiene can cause masks to become reservoirs for antimicrobial-resistant bacteria, increasing the risk of hospital-acquired infections (HAIs) and community-associated transmission (22,23). Regular mask change, careful handling, and adherence to infection prevention practices are essential low-cost, high-impact methods for mitigating AMR spread and HAI incidence (23).

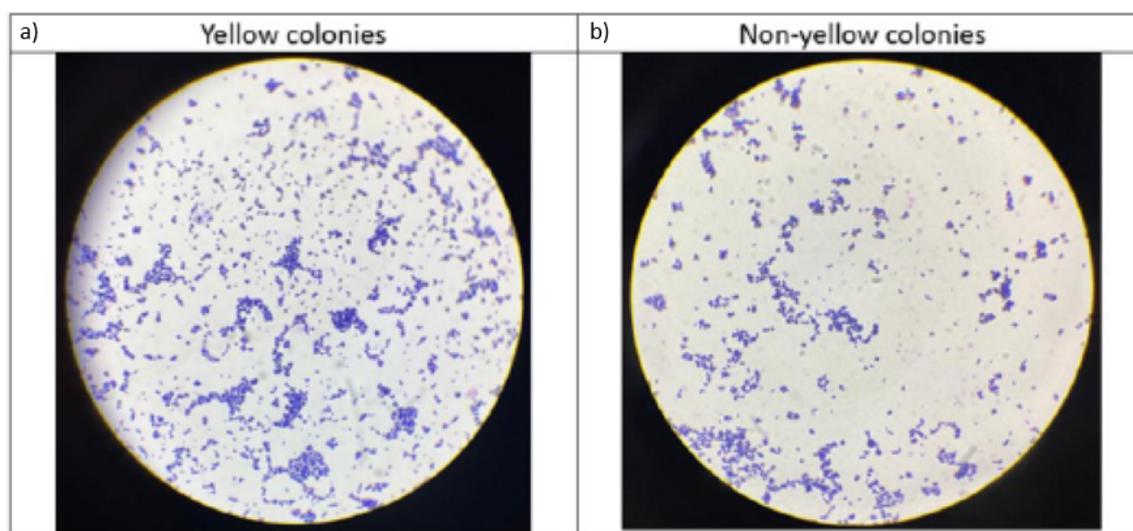


Fig 3: Photomicrograph of Gram stain of yellow (a) and non-yellow colonies (b) cultured on MSA agar under 100x magnification.

It is important to acknowledge that this study had several limitations that should be addressed. First, the area of interest in this study was only the face-side of the face mask because it is reported in other study that the colony count found on the face-side of face mask is around 13 times higher than the outer side of face

mask (8). Furthermore, improper use of face masks can undermine the protective effect of masks and possibly raise the risk of self-contamination, like frequent touching, wearing soiled masks, or improper storage. Such challenges highlight the necessity of thorough instruction on mask hygiene, regular observation, and institutional assistance, such as the provision of hygienic mask substitutes (8,16).

4. Conclusion

This study found an increase in bacterial load on the face-side of masks worn between four and seven hours, with significantly more total and yellow colonies on masks worn for seven hours. The results provide valuable insights into bacterial accumulation on face masks over different time

intervals, which is particularly relevant for preclinical medical students as they advance in their medical training. Understanding these dynamics is crucial for ensuring both patient and personal safety, emphasizing the importance of proper mask usage and adherence to hygiene protocols in preventing nosocomial infections. Further research is needed to determine the optimal safe duration for mask use to

reduce the risk of self-contamination.

Ethics Committee with Ref. no. FOMBN/EC/18/23/02.

Funding and Ethical Approval

The authors of this study gratefully acknowledge the generous financial support provided by MAHSA University with Grant ID: RP175-05/21. Ethical approval was also obtained by MAHSA university

Acknowledgements

We would like to thank the preclinical undergraduate medical students of MAHSA University for their participation in this study.

References

1. Andrejko KL, Pry JM, Myers JF, Fukui N, DeGuzman JL, Openshaw J, et al. California COVID-19 Case-Control Study Team. Effectiveness of face mask or respirator use in indoor public settings for prevention of SARS-CoV-2 infection - California, February-December 2021. *MMWR Morb Mortal Wkly Rep.* 2022 Feb 11;71(6):212-6. doi: 10.15585/mmwr.mm7106e1.
2. Howard J, Huang A, Li Z, Tufekci Z, Zdimal V, van der Westhuizen HM, et al. An evidence review of face masks against COVID-19. *Proc Natl Acad Sci U S A.* 2021 Jan 26;118(4):e2014564118. doi: 10.1073/pnas.2014564118.
3. Davies A, Thompson KA, Giri K, Kafatos G, Walker J, Bennett A. Testing the efficacy of homemade masks: would they protect in an influenza pandemic? *Disaster Med Public Health Prep.* 2013 Aug;7(4):413-8. doi: 10.1017/dmp.2013.43.
4. Lai AC, Poon CK, Cheung AC. Effectiveness of facemasks to reduce exposure hazards for airborne infections among general populations. *J R Soc Interface.* 2012 May 7;9(70):938-48. doi: 10.1098/rsif.2011.0537. Epub 2011 Sep 21.
5. World Health Organization. (2020). Rational use of personal protective equipment (PPE) for coronavirus disease (COVID-19): interim guidance, 19 March 2020. World Health Organization. <https://iris.who.int/handle/10665/331498>. License: CC BY-NC-SA 3.0 IGO.
6. Kaur T, Kaur S. A multi-center, cross-sectional study on the prevalence of facial dermatoses induced by mask use in the general public during the COVID-19 pandemic. *Our Dermatol Online.* 2022;13(1):1-5.
7. Chughtai AA, Stelzer-Braid S, Rawlinson W, Pontivivo G, Wang Q, Pan Y, et al. Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers. *BMC Infect Dis.* 2019 Jun 3;19(1):491. doi: 10.1186/s12879-019-4109-x.
8. Park AM, Khadka S, Sato F, Omura S, Fujita M, Hashiwaki K, et al. Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Sci Rep.* 2022 Jul 18;12(1):11361. doi: 10.1038/s41598-022-15409-x.
9. Delanghe L, Cauwenberghs E, Spacova I, De Boeck I, Van Beeck W, Pepermans K, et al. Cotton and Surgical Face Masks in Community Settings: Bacterial Contamination and Face Mask Hygiene. *Front Med (Lausanne).* 2021 Sep 3;8:732047. doi: 10.3389/fmed.2021.732047.
10. Zhiqing L, Yongyun C, Wenxiang C, Mengning Y, Yuanqing M, Zhenan Z, et al. Surgical masks as source of bacterial contamination during operative procedures. *J Orthop Translat.* 2018 Jun 27;14:57-62. doi: 10.1016/j.jot.2018.06.002.
11. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, Conlan S, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. *Sci Transl Med.* 2017 Jul 5;9(397):eaal4651. doi: 10.1126/scitranslmed.aal4651.
12. Radonovich LJ Jr, Cheng J, Shenal BV, Hodgson M, Bender BS. Respirator tolerance in health care workers. *JAMA.* 2009 Jan 7;301(1):36-8. doi: 10.1001/jama.2008.894. PMID: 19126810.
13. Amir AB, Dahalan FA, Anjarwati L, Kurniawan WR. Assessment of microbiological growth on surgical face mask. *Environmental and Toxicology Management.* 2022 Dec 20;2(3):6-9. doi: 10.33086/etm.v2i3.3557.
14. Li H, Li L, Chi Y, Tian Q, Zhou T, Han C, Zhu Y, Zhou Y. Development of a standardized Gram stain procedure for bacteria and inflammatory cells using an automated staining instrument. *Microbiologyopen.* 2020 Sep;9(9):e1099. doi: 10.1002/mbo3.1099. Epub 2020 Jun 27. PMID: 32592452; PMCID: PMC7520987.
15. Abdeen EE, Mousa WS, Abdelsalam SY, Heikal HS, Shawish RR, Nooruzzaman M, Soliman MM, Batiha GE, Hamad A, Abdeen A. Prevalence and Characterization of Coagulase Positive *Staphylococci* from Food Products and Human Specimens in Egypt. *Antibiotics (Basel).* 2021 Jan 14;10(1):75. doi: 10.3390/antibiotics10010075. PMID: 33466771; PMCID: PMC7829985.
16. Chiera, S., Bosco, F., Mollea, C. *et al.* *Staphylococcus epidermidis* is a safer surrogate of *Staphylococcus aureus* in testing bacterial filtration efficiency of face masks. *Sci Rep* 13, 21807 (2023). <https://doi.org/10.1038/s41598-023-49005-4>
17. Urquía-Cutillas S, Gómez-Lus ML, Sevillano D, González N, Alou L. Do face masks increase the rate of the *Staphylococcus aureus* nasal carriers? *Rev Esp Quimioter.* 2023 Oct;36(5):549-551. doi: 10.37201/req/082.2023. Epub 2023 Sep 7. PMID: 37675477; PMCID: PMC10586734.
18. Shimamura, Y., Ozaki, M., Shinya, M. *et al.* Factors influencing bacterial viability on face masks and bactericidal effect of disinfection methods. *Sci Rep* 15, 24357 (2025). <https://doi.org/10.1038/s41598-025-09204-7>.
19. Neary M, Quan K, Tjoa T, Bittencourt CE, Huang SS, Uy C. Did universal masking during the

- COVID-19 pandemic reduce MRSA and MSSA acquisition in the NICU? *Infection Control & Hospital Epidemiology*. Published online 2025:1-6. doi:10.1017/ice.2025.10209
20. Maheswary T, Nurul AA, Fauzi MB. The Insights of Microbes' Roles in Wound Healing: A Comprehensive Review. *Pharmaceutics*. 2021 Jun 29;13(7):981. doi: 10.3390/pharmaceutics13070981. PMID: 34209654; PMCID: PMC8308956.
21. Kisielinski Kai, Wojtasik Barbara, Zalewska Aleksandra, Livermore David M., Jurczak-Kurek Agata. The bacterial burden of worn face masks-observational research and literature review. *Frontiers in Public Health*, Volume 12 – 2024. DOI=10.3389/fpubh.2024.1460981.
22. Martí, M., Tuñón-Molina, A., Aachmann, F. L., Muramoto, Y., Noda, T., Takayama, K., & Serrano-Aroca, Á. (2021). Protective Face Mask Filter Capable of Inactivating SARS-CoV-2, and Methicillin-Resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Polymers*, 13(2), 207. <https://doi.org/10.3390/polym13020207>
23. Salachas C, Le Hingrat Q, Haviari S, Valdes A, Mackiewicz V, Lolom I, Fidouh N, Visseaux B, Bouzid D, Descamps D, Lucet JC, Charpentier C, Kernéis S. Associations between hospital structure, infection control and incidence of hospital-acquired viral respiratory infections: a 10-year surveillance study. *Antimicrob Resist Infect Control*. 2025 Apr 11;14(1):28. doi: 10.1186/s13756-025-01543-4. PMID: 40217353; PMCID: PMC11992881.
24. Nightingale M, Mody M, Rickard A, Cassone M. Bacterial contamination on used face masks in healthcare personnel. *Antimicrobial Stewardship & Healthcare Epidemiology*. 2022;2(S1):s86-s87. doi:10.1017/ash.2022.220
25. Justin LYS, Yew YW. Facial dermatoses induced by face masks: A systematic review and meta-analysis of observational studies. *Contact Dermatitis*. 2022 Dec;87(6):473-484. doi: 10.1111/cod.14203. Epub 2022 Sep 2. PMID: 35980367; PMCID: PMC9539048.