Malaysian Journal of Microbiology, Vol 19(1) 2023, pp. 87-91
DOI: http://dx.doi.org/10.21161/mjm.220014

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

SHORT COMMUNICATION

Effects of chewing gum on mask contamination

Sung-Yoon Won[1] and Dong-Eun Lee[2]*

[1]Department of Occupational Therapy, Semyung University, Chung-Buk, South Korea.
[2]Department of Dental Hygiene, Ulsan College, Ulsan, South Korea.
Email: rhie1010@naver.com

Received 14 July 2022; Received in revised form 21 December 2022; Accepted 27 December 2022

ABSTRACT

Aims: The aim of this study was to evaluate whether chewing gum affects mask contamination.
Methodology and results: Two groups of participants were requested to wear a mask for 15 min with (experimental group) or without (control group) chewing gum. Then, masks were collected and CFU calculation and 16S rDNA sequencing was performed. We found that temperature, humidity and bacterial CFU inside of the mask significantly increased when wearing a mask while chewing gum. *Staphylococcus epidermidis* was found in both groups. *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus oralis*, *Streptococcus parasanguinis* and *Bacillus wiedmannii* were found in only the experimental group.
Conclusion, significance and impact of study: Chewing gum significantly increased the temperature, humidity and bacterial CFU inside the mask. *Staphylococcus epidermidis*, *S. aureus*, *S. haemolyticus*, *S. oralis*, *S. parasanguinis* and *B. wiedmannii* were detected inside the mask after chewing gum.

Keywords: Chewing gum, gum, mask contamination, mastication

INTRODUCTION

People chew gum to relieve tension, improve concentration, prevent drowsiness and reduce halitosis (Wilkinson *et al*., 2002; Aslani and Jalilian, 2013, Muniz *et al*., 2017; Sekine *et al*., 2020). Chewing gum can also increase cognitive ability and improve work efficiency (Allen and Smith, 2015). Sometimes chewing gum is encouraged to reduce stress (Kubo *et al*., 2015). In a previous study, chewing gum reduced the required time for the Stroop test, suggesting one of the many positive aspects of chewing gum (Won and Lee, 2021).

Due to the COVID-19 pandemic, wearing a mask has become essential (Honein *et al*., 2020). A previous study showed that used masks were contaminated by increased temperature and humidity, especially as the wearing time increased. In addition, the contamination rate increased with the conversation, suggesting that the outflow of saliva from the oral cavity due to conversation can increase the contamination rate of the mask (Lee, 2022).

Since it is known that mastication can cause saliva outflow from the oral cavity (Gavião *et al*., 2004), we hypothesized that chewing gum could increase saliva's outflow and increase the mask's contamination rate. Therefore, in this study, the effect of chewing gum on mask contamination was evaluated.

MATERIALS AND METHODS

Participants

To calculate the sample size, a test power calculation was completed (effect size d=0.5; alpha error=0.05; power=0.95, G*Power 3.1.9.7). After explaining the purpose of the study, participants (n=45, mean age 27.2 ± 2.3, no medical history, free of medication) were recruited with written informed consent.

Questionnaire examination

The participants responded to a questionnaire that included questions about whether they washed their faces, brushed their teeth and/or put on makeup before wearing a mask. Participants who stated they did not wash their face, not brush their teeth or put on makeup before wearing a mask were excluded.

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(1) 2023, pp. 87-91
DOI: http://dx.doi.org/10.21161/mjm.220014

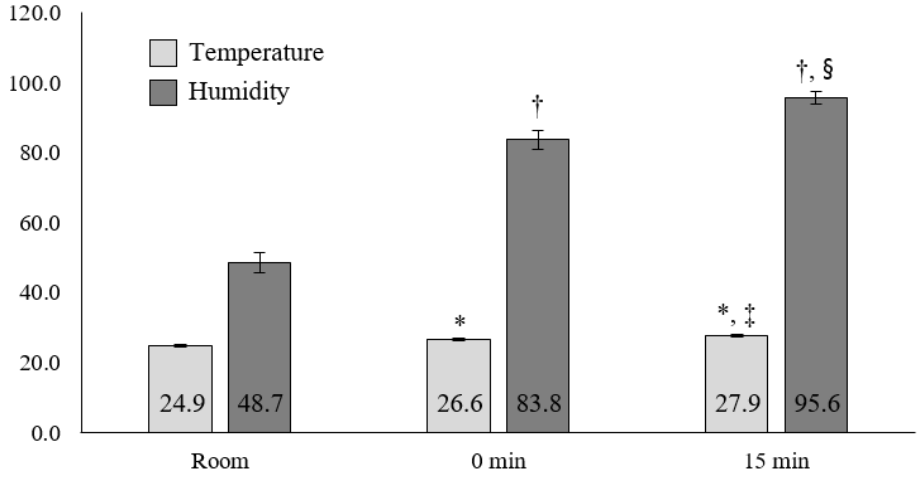

Figure 1: Temperature and humidity inside the mask. *Indicates significant differences compared to room temperature (p<0.01); † indicates significant differences compared to room humidity (p<0.01); ‡ indicates significant differences compared to 0 min (p<0.01); § indicates significant differences compared to 0 min (p<0.01).

Experimental protocols

The KF-AD masks (Hwa-Jin, Seoul, South Korea) and a piece of xylitol gum (Lotte, Seoul, South Korea) were distributed to all participants. Before the start of the experiment, all masks were sterilized with ultraviolet (UV) light for 40 min. To avoid contamination, all participants washed their hands and were instructed to only touch the ear loops while placing the mask on their faces. If contamination occurred, the mask was discarded and a new one was distributed. All participants chewed gum for 15 min with a mask on. Then the masks were collected and placed in a sterile zipper bag and sent to the laboratory. The temperature and humidity inside of the masks at 0 and 15 min were measured (Thermo-Hygrometer Pocket/Digital, DAIHAN, South Korea).

Colony forming unit (CFU) measurement

After collecting the mask, a 1 cm × 1 cm square piece from the center of the mask was cut out and the inner layer was separated. These separated pieces were placed in an Eppendorf tube containing 1 mL of Luria Bertani broth, vortexed for 1 min and then incubated overnight at 37 °C. After dilution (10^{-1} to 10^{-7}), 100 µL was plated onto plate count agar (PCA) plates. Bacterial CFU was calculated after the overnight incubation.

Bacteria identification

In PCA agar plate, colonies were picked randomly and subcultured 3 times. Polymerase chain reaction (PCR) was implemented with paired primer set (Forward primer: 27F: 5'-AGA GTT TGA TCM TGG CTC AG-3', Reverse primer: 1492R: 5'-GGT TAC CTT GTT ACG ACT TC-3'). The reaction mixtures were subjected to 30 cycles of denaturation and annealing at 50 °C in an automated thermal cycler (ABI simpliamp thermal cycler, Applied Biosystems, ThermoFisher, USA). After PCR, the PCR products were electrophoresis, then purified (DNA Purification Kit, LaboPass, Seoul, South Korea). Finally, the PCR products were analyzed with automated sequencing using a BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Beverly, MA), ABI 3730XL sequencing machine (Applied Biosystems) and Sequencing analysis 5.2 program (Gene Codes Corp., Ann Arbor, MI). The newly aligned 16S rDNA sequences were compared with the bacterial genes deposited in the GenBank database (National Center for Biotechnology Information, Bethesda, MD) (Zheng *et al*., 2000) and bacterial strains with more than 99% matches were searched using Basic Local Alignment Search Tool for nucleotides (BLAST; National Center for Biotechnology Information). After 16S rDNA sequences analysis, all identified bacteria were listed, and the distribution of each type of bacteria was calculated as a percentage (%).

Data analysis

SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Data were evaluated by one-way analysis of variance (ANOVA) followed by paired t-test. The results of all experiments are represented as the mean ± standard error (SE). p<0.01 and 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Compared to room temperature (24.9 °C), the temperature inside of the mask significantly increased when wearing a mask (26.6 °C, p<0.01) and after 15 min of chewing gum (27.9 °C, p<0.01). Compared to room humidity (48.7%), the humidity inside of the mask significantly increased when wearing a mask (83.8%, p<0.01) and after 15 min of chewing gum (95.6%, p<0.01) (Figure 1).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 19(1) 2023, pp. 87-91
DOI: http://dx.doi.org/10.21161/mjm.220014

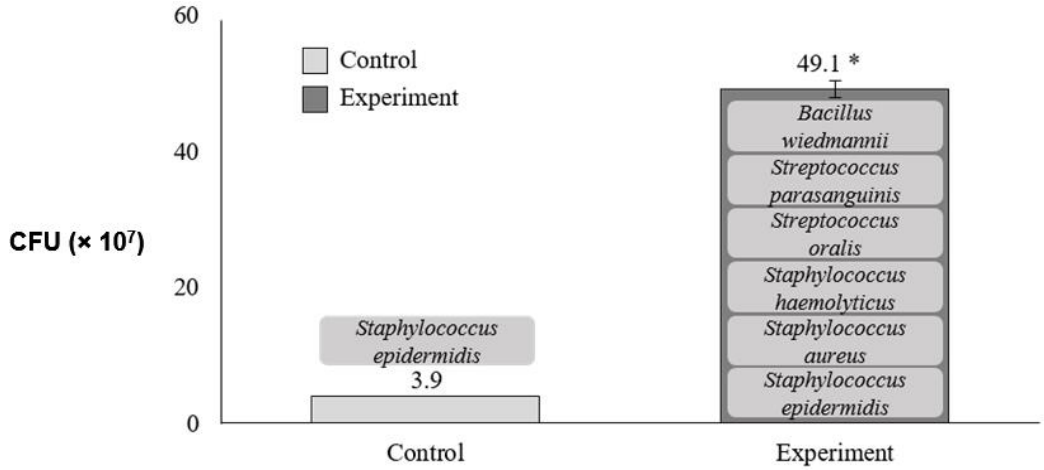

Figure 2: Bacterial CFU inside the mask. *Indicates significant differences compared to control (p<0.01).

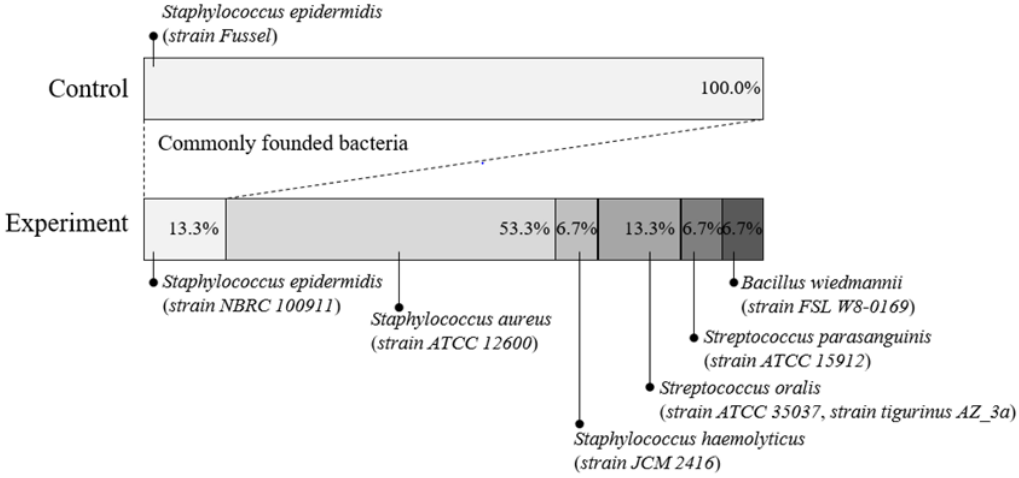

Figure 3: Bacterial species inside the mask.

Compared to the control (3.9 × 10^7), the bacterial CFU significantly increased (49.1 × 10^7) after 15 min of chewing gum (p<0.01) (Figure 2). In addition, the bacterial species present in the control and experimental groups were different. In the control group, *Staphylococcus epidermidis* (*S. epidermidis*, strain Fussel) was found. In the experimental group, *S. epidermidis* (strain NBRC 100911), *S. aureus* (strain ATCC 12600), *S. haemolyticus* (strain JCM 2416), *S. oralis* (strain ATCC 35037 and tigurinus AZ_3a), *S. parasanguinis* (strain ATCC 15912) and *B. wiedmannii* (strain FSL W8-0169) were found. *S. epidermidis* was found in both groups, but the strain was different.

Temperature, humidity, pH, nutrient content and the presence or absence of oxygen are major factors for bacterial growth (Cha, 2021). Homeostatic environments, such as consistently warm temperatures and high humidity, can stimulate bacterial growth. The oral cavity contains many nutrients (Cha, 2021). It has been reported that wearing a mask maintains a warm temperature and high humidity inside the mask (Lee, 2022). Therefore, the inside of a mask presents ideal conditions for bacterial growth. Our results showed that the temperature and humidity inside the mask did increase compared to the environment (Figure 1).

In a previous study, the contamination rate inside the mask was also affected by wearing time and conversation, perhaps due to increased saliva outflow (Lee, 2022). In addition, previous studies showed that mastication could cause saliva outflow (Gavião *et al*., 2004). Therefore, we hypothesized that the mastication of chewing gum increases saliva outflow and thus can increase the contamination inside a mask. Compared to the control, we found that chewing gum for 15 min increased bacterial CFU in the experimental group (Figure 2).

We performed 16S rDNA sequencing to identify the bacterial species, which were found to be different between both groups. *S. epidermidis* was found in both groups, but the strain was different. In control, only the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Fussel strain was detected, but NBRC 100911 was detected in the experimental group. Unlike the control group, various types of bacteria were found inside of the mask in the experimental group: *S. epidermidis* (found in 13.3% of the total samples), *S. aureus* (53.3%), *S. haemolyticus* (6.7%), *S. oralis* (13.3%), *S. parasanguinis* (6.7%) and *B. wiedmannii* (6.7%). In *S. oralis*, strain ATCC 35037 and tigurinus AZ_3a were found at 50.0% each, *Staphylococcus* was found in 73.3% of samples, *Streptococcus* at 20.0% and *Bacillus* at 6.7% (Figure 3).

Staphylococcus epidermidis, S. aureus and *S. haemolyticus* are Gram-positive, facultative anaerobic bacteria that are part of the normal human flora. *S. epidermidis* (Schleifer and Kloos, 1975) is not usually pathogenic, but infections caused by these bacteria are generally nosocomial. *S. aureus* (Masalha *et al*., 2001) *and S. haemolyticus* (Schleifer and Kloos, 1975) can also become opportunistic pathogens. Since the mask directly contacts the facial skin, it is a high possibility that the normal bacteria found on the skin can be found in the inner layer of the mask.

S. oralis and *S. parasanguinis* are Gram-positive, facultative anaerobic bacteria. *S. oralis* (Do *et al*., 2009) is found in the oral cavity and is classified as a member of the *Streptococcus mitis* group, which are opportunistic pathogens. *S. parasanguinis* (Chen *et al*., 2019) is one of the major early colonizers of dental surfaces in the oral cavity and is classified as a member of the *Streptococcus viridans* group. These bacteria, commonly found in the oral cavity, were found inside the mask. This suggests that chewing gum can cause a leak of bacteria from the oral cavity and contaminate the inside of the mask.

Interestingly, *B. wiedmannii*, which is rarely found in humans, was detected in our samples. *B. wiedmannii* (Miller *et al*., 2016) is a Gram-positive, facultative anaerobic bacterium. Before the start of the experiment, the mask was sterilized with UV light. Autoclaving is a reliable method to kill bacteria, but it was not selected because it can reduce the effectiveness of the mask filter. It is known that UV sterilization for more than 40 min can kill most bacteria (Cha, 2021), including spore-forming bacteria, but handling the mask may have caused contamination in this study. It is also possible that the mask may have been contaminated by an unexpected route.

The results showed that chewing gum could increase the contamination rate inside of the mask by causing the outflow of oral bacteria.

A limitation of the study was that only one type of mask was tested and that the participants' respiratory rate and the duration of chewing gum varied among participants.

CONCLUSION

Chewing gum significantly increased the temperature, humidity and bacterial CFU inside of the mask. *Staphylococcus epidermidis*, *S. aureus*, *S. haemolyticus*, *S. oralis*, *S. parasanguinis* and *B. wiedmannii* were detected inside of the mask after chewing gum.

ACKNOWLEDGEMENTS

This work was supported by the 2022 Research Fund of Ulsan College.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Allen, A. P. and Smith, A. P. (2015). Chewing gum: Cognitive performance, mood, well-being, and associated physiology. *BioMed Research International* **2015, Article ID 654806.**

Aslani, A. and Jalilian, F. (2013). Design, formulation and evaluation of caffeine chewing gum. *Advanced Biomedical Research* **2, 72.**

Cha, J. H. (2021). Oral Microbiology. Medical Education Publishing, Seoul. **pp. 23-28.**

Chen, Q., Wu, G., Chen, H., Li, H., Li, S., Zhang, C. *et al*. (2019). Quantification of human oral and fecal *Streptococcus parasanguinis* by use of quantitative real-time PCR targeting the *groEL* gene. *Frontiers in Microbiology* **20, 2910.**

Do, T., Jolley, K. A., Maiden, M. C., Gilbert, S. C., Clark, D., Wade, W. G. *et al.* (2009). Population structure of *Streptococcus oralis*. *Microbiology* **155(8), 2593-2602.**

Gavião, M. B. D., Engelen, L. and van der Bilt, A. (2004). Chewing behavior and salivary secretion. *European Journal Oral Sciences* **112(1), 19-24.**

Honein, M. A., Christie, A., Rose, D. A., Brooks, J. T., Meaney-Delman, D., Cohn, A. *et al.* (2000). Summary of guidance for public health strategies to address high levels of community transmission of SARS-CoV-2 and related deaths. *Morbidity and Mortality Weekly Report* **69(49), 1860-1867.**

Kubo, K. Y., Iinuma, M. and Chen, H. (2015). Mastication as a stress-coping behavior. *BioMed Research International* **2015, Article ID 876409.**

Lee, D. E. (2022). Bacterial contamination on the inner surface of the masks used by clinicians in a dental clinic. *Dental and Medical Problems* **59(2), 177-185.**

Masalha, M., Borovok, I., Schreiber, R., Aharonowitz, Y. and Cohen, G. (2001). Analysis of transcription of the *Staphylococcus aureus* aerobic class Ib and anaerobic class III ribonucleotide reductase genes in response to oxygen. *Journal of Bacteriology* **183(24), 7260-7272.**

Miller, R. A., Beno, S. M., Kent, D. J., Carroll, L. M., Martin, N. H., Boor, K. J. *et al.* (2016). *Bacillus wiedmannii* sp. nov., a psychrotolerant and cytotoxic *Bacillus cereus* group species isolated from dairy foods and dairy environments. *International Journal of Systematic and Evolutionary Microbiology* **66(11), 4744-4753.**

Muniz, F. W. M. F., Friedrich, S. A., Silveira, C. F. and Rösing, C. K. (2017). The impact of chewing gum on

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

halitosis parameters: A systematic review. *Journal of Breath Research* **11(1), 014001.**

Schleifer, K. H. and Kloos, W. E. (1975). Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. *International Journal of Systematic and Evolutionary Microbiology* **25(1), 50-61.**

Sekine, N., Okada-Ogawa, A., Asano, S., Takanezawa, D., Nishihara, C., Tanabe, N. *et al.* (2020). Analgesic effect of gum chewing in patients with burning mouth syndrome. *Journal of Oral Science* **62(4), 387-392.**

Wilkinson, L., Scholey, A. and Wesnes, K. (2002). Chewing gum selectively improves aspects of memory in healthy volunteers. *Appetite* **38(3), 235-236.**

Won, S. Y. and Lee, D. E. (2021). Effects of chewing gum stimuli on general and emotional Stroop test. *Current Research in Nutrition and Food Science* **9(2), 479-486.**

Zheng, Z., Schwartz, S., Wagner, L. and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7(1-2), 203-214.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538