Clinical and Experimental Health Sciences

The Used Masks of The Food Industry Employees During the Covid-19 Pandemic: Did the Mask Promote Other Diseases While Protecting from the Coronavirus? A Survey Study Supported by Microbiological Data

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Received: 13.07.2023 **Accepted:** 07.08.2023

ABSTRACT

Objective: Use of disposable face masks helps prevent infection by airborne pathogens. The effectiveness of such masks in excluding diseases and contaminants depends on many factors. As a result of misuse, mask loses its protective role and becomes a source of microbial contamination. It is aimed to investigate the attitudes of food workers towards use of masks in proportion to the bacterial load and microorganism species in the masks they wear.

Method: Total aerobic mesophilic, Yeast-mould and Coliform counts were determined as log colony forming units per mL. Phenotypically different colonies grown on three different agar plates were purified and fresh cultures were classified using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Results: In this study, bacterial contamination was found at different levels on all tested (103) disposable face masks. The screened bacterial pathogens by MALDI-TOF MS Bacillus cereus was detected at the highest level 17.86%, Enterobacter cloacae, Klebsiella oxytoca, Kurthia gibsonii and Pseudomonas aeruginosa with rates 14.29%, 10.72%, 7.14%, and 7.14%, respectively. Long-term working, inappropriate mask usage, poor hygiene attitudes of employees, and the fact that staff wore the mask out of need rather than for its protective advantage all signal that the investigated masks had low microbiological quality.

Conclusion: The findings show that because most masks used by food industry employees to protect themselves from COVID-19 and avoid infecting others contain bacteria of intestinal origin, serious health problems may occur in both employees who use contaminated masks and consumers who consume food contaminated by mask contamination.
Keywords: COVID-19; face masks; MALDI-TOF MS; food industry employees; microbiology

1. INTRODUCTION

The novel coronavirus, which started as a localized zoonotic disease outbreak in China – December 2019 – spread rapidly to many other countries, causing it to be declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (1). The pandemic has greatly affected social life in every sense. For this reason, various guidelines and resources have been developed at local, national, and international levels, both in the private and public sectors. Almost all countries, especially those most affected by the epidemic, have taken various precautions to reduce the epidemic. Full or partial quarantine practices, travel restrictions, and mandatory use of masks in public areas are among the foremost of these measures (2).

Food industry is one of the most important sections of a country's critical infrastructure along with health, energy, and communication (2). Nutrition, which is the most basic need of people during the pandemic, should continue to be maintained at a normal life level (3). Therefore, during pandemic, the food industry continues to struggle with some challenges such as preventing disruptions from the supply chain (4), meeting consumer demand, protecting the workforce while ensuring food safety, and not disrupting consumer confidence (5). Personal hygiene and health are essential to maintaining a hygienic food processing environment. The implementation of Food Safety Management Systems (FSMS) based on Hazard Analysis and Critical Control Point (HACCP) principles provides control of

DOI: 10.33808/clinexphealthsci.1326608

food safety (6). Studies indicate that the factors potentially affecting the risk of infection include difficulties with physical distance, hygiene, crowded living, and transportation conditions in the workplace (7, 8). As stated by the European Food Safety Authority (9) there is no evidence that food poses a risk to public health in Europe. Infection occurs through respiratory droplets formed by sneezing, coughing, or breathing in infected persons (10). Respiratory droplets do not stay suspended in the air, but quickly fall on floors and surfaces. Although it has greatly affected social life, there seems to be no report that COVID-19 is transmitted through food consumption. For these reasons, the risk of COVID-19 transmission increases in the form of touching a contaminated surface or touching the mouth, nose, and eyes (11).

The most common way to avoid the risk of contamination is the use of Personal Protective Equipment (PPE) (12). When used correctly, PPE, together with proper hygiene and hand washing practices, help reduce the spread of cross-infection (COVID-19) and cross-contamination (food safety) (1, 11). PPE commonly used in the food industry may include face masks, face shields, gloves, aprons, bonnets, and work shoes. Since SARS-CoV-2 is a respiratory virus, the mask has been put in the first place in terms of precautions among other PPE. WHO has stated that the most effective ways to prevent an epidemic and minimise its spread are the use of surgical masks, along with hand hygiene and other preventive measures (13).

Face masks have previously been used by paramedics and infected people, especially in epidemics such as the 2009 influenza pandemic, avian flu, Middle East respiratory syndrome coronavirus (MERS-coronavirus) and Ebola virüs (14). The most used disposable surgical and N95 type masks are produced to filter droplets containing microorganisms expelled from the mouth and nose. However, to prevent the spread of airborne pathogens, some factors should be considered, especially the selection of masks with different pore sizes and the types (15). The generally accepted assumption is that use of masks, both medical and non-medical, is safe, but this should be closely monitored and studied in detail. Studies on mask effectiveness are available in the literature (16, 17). Masks, in continuous and correct use, reduces the rate of virus infection (18) but it is less effective in protecting the user from being infected (19). The fact that microorganisms in human saliva and exhaled breath can pose a biosafety problem is often overlooked, especially when worn for too long, not stored properly, or reused without proper disinfection. Human saliva contains around 100 million bacterial cells per milliliter. These bacteria include pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Klebsiella pneumoniae*, *Neisseria*, *Prevotella* and *Veillonella* spp. (16). Cotton contained in the masks a moisture-retaining property, making cotton masks suitable for microbial contamination. For this reason, reused cotton masks increase the risk of respiratory transmission (20). Studies show that surgical masks may not be sufficient to protect people from airborne pathogens, and moreover, these masks may be a source of infection (21). Surgical face masks worn for more than 4 hours show higher contamination than those worn less often. On the other hand, the use of face masks affects individual and social life for reasons such as discomfort, respiratory distress, headache, skin acne and difficulty in communication (16).

We hypothesized that masks used to minimise bacterial contamination from the mouth, nose, and face can be a source of contamination when worn for a long time, reused several times, and when used masks come into contact anywhere. The present study examines the role of the masks in personal and community hygiene and their role as a potential source of bacterial contamination. For this reason, it is aimed to investigate the hygiene status of food workers, their attitudes towards mask use, the bacterial load proportional to the duration of use in the masks they wear, the microorganism types in the contaminated masks and their correlation with survey and microbiological results.

2. METHODS

2.1. Sampling

The research was carried out between 01-31 December 2021 in the city center of Burdur, Türkiye (37.7183° N, 30.2823° E). The study aimed to determine the status and microbiological quality of the masks used by food industry employees. Prior to sampling, the permission of the owner of the enterprise was obtained, and then the participation of food industry employees was determined on a voluntary basis. Before starting the research, the employees were informed about the study and questionnaire were applied to the employees who read and accepted the voluntary consent form. Attention was paid to the fact that participants were between 18-65 ages without any psychological problems, and a total of 103 participants were subject of the research. The protocol (GO 2021/422) was approved by the Non-Interventional Clinical Research Ethics Committee of Burdur Mehmet Akif Ersoy University.

2.2. Collection of Research Data

The survey data were prepared by the researchers after the relevant literature was searched and the data were collected by face-to-face interview method. The questionnaire form consists of 3 sections in total, including the socio-demographic information of the food industry employees, the questions about the workplace and working conditions, and the questions prepared to determine the use of masks. Individuals between the ages of 18-65 who participated in the research were given general information about the research, and the volunteer consent form was read to the participants who wanted to participate in the study and their approval was obtained.

2.3. Microbiological Evaluation

2.3.1. Bacterial isolation

The masks collected throughout the survey were sent to the laboratory in closed sterile stomacher bags. Initially, all the samples were enriched onto buffered peptone water for 24 hours at 37 °C. Appropriate serial dilutions were prepared in 0.1% peptone water solution, and bacterial populations were counted. Total aerobic mesophilic counts were enumerated on plate count agar (*Oxoid CM325*) incubated at 30±1 ºC for 48 h. Yeast and mould counts were enumerated on potato dextrose agar (*Difco B 13*). Plates were incubated at 22±1 ºC and evaluated after 5 days. Coliform counts were enumerated on violet, red bile agar (*Difco B12*). Plates were incubated at 30±1 ºC and evaluated after 24 h. Inoculated plates in duplicate were incubated and at the end of incubation only plates containing 30-300 colonies were evaluated. Microorganism counts of samples were expressed as log colony forming units per mL (log_{10} CFU/mL).

2.3.2. MALDI-TOF MS identification of colonies

Phenotypically different colonies grown on three different agar plates were purified by spreading on Tryptic Soy Agar (TSA) (*Merck 1.05458*). Pure and fresh cultures were classified using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex® LT/SH, Bruker Daltonik, Bremen, Germany) and the flexControl and MALDI Biotyper® Compass software packages (Bruker Daltonik). After the pure cultures were obtained, formic acid + ethanol extraction processes were started for MALDI TOF MS analysis. Picked single colonies were transferred to a MALDI-plate and covered with 1 µL of 70% formic acid and allowed to dry at room temperature. The plates were overlayed with 2 µL of α-Cyano-4-hydroxycinnamic acid (HCCA) matrix solution and placed into the MALDI-TOF MS. Samples were analysed using the Bruker Daltonics flexControl-MicroFlex LT system. The spectra captured with the Flex control software program were compared with the MALDI Biotyper Real-Time Classification (RTC) software and the diagnosis process was completed. Measurements were continued until the bacterium was clearly identified. The meaning of the resulting scores was evaluated according to the manufacturer's instructions. In this context, a score between 2.300-3.000 (green color) indicates highly probable species identification; a score between 2.000-2.299 (green color) indicates secure genus identification, probable species identification; a score between 1.700-1.999 (yellow color) indicates probable species identification; and a score between 0.000-1.699 (red color) indicates not reliable identification (22).

2.4. Statistical Analysis

The data obtained in the study were evaluated using Statistical Package for The Social Sciences (SPSS Inc., Chicago, IL, USA) 26.0 package program. Number (n) and percentage (%) rates are given for categorical data and mean (X) standard deviation (SD) values are given for numerical data. Mean (X) standard error (SE) is given as the mean of the number of diseases that participants had in the last year. In statistical analysis, frequency in socio-demographic information, independent sample t-test according to the characteristics of the variable in numerical data, and one-way analysis of variance (ANOVA) were used. In one-way analysis of variance, differences between groups were calculated using Tukey's multiple comparison test. $p< .05$ was considered statistically significant.

3. RESULTS

3.1. Socio-demographic Data of the Participants

The results of the survey conducted with the food industry employees and the microbiological analysis of the masks used by the employees were examined. Accordingly, when the age group distributions of the individuals participating in the study are examined, it is seen that 37.9% (n=39) are between the ages of 18-24 and 21.4% (n=22) are between the ages of 25-34. It was determined that the mean of individuals in the 55-65 age range was 5.8% (n=6), the group with the lowest distribution among all age groups. The distribution of age groups showed similarity in both genders; it is seen that the number of individuals in the 25-34 age range is low only in the female gender (Table 1).

It was determined that the participants were sick on average 1.9±0.2 times in the past year, and 55.3% (n=57) of the diseases were respiratory system diseases and 16.5% (n=17) were COVID-19. 25 people (24.3%) stated that they had not had any disease in the last year. It was found that 60.2% (n=62) of the people working in food businesses are permanent employees, 25.2% (n=26) are business owners or partners and 14.6% (n=15) are part-time employees. It was determined that 33.0% (n=34) of the individuals participating in the study kept the masks they used in their pockets, and 16.5% (n=17) kept their chins. It was observed that only 18.4% (n=19) of the individuals did not store the mask they used or used a new mask. 41.7% (n=43) of the individuals participating in this study stated that they used a mask for 9-24 hours, and 23.3% (n=24) used it for more than 1 day. When the individuals participating in the study were questioned whether they used masks or other protective equipment used by others, it was seen that 93.2% (n=96) answered no. Although the rate of individuals who say yes is low, it is estimated that they are from individuals with family ties. When asked whether masks protect the person from diseases, 51.5% (n=53) of the individuals participating in the study answered no; It was observed that 47.5% (n=49) answered yes and 1 person did not express an opinion.

***Table 1.** Socio-demographic data of the participants*

	Female		Male		Total	
	X	SE	X	SE	X	SE
Number of diseases in the last year						
	2.5	0.3	1.5	0.2	1.9	0.2
	n	%	n	%	n	%
Age distributions of participants						
18-24	21	47.7	18	30.5	39	37.9
25-34	5	11.4	17	28.8	22	21.4
35-44	8	18.2	13	22.0	21	20.4
45-54	8	18.2	7	11.9	15	14.6
55-65	2	4.5	4	6.8	6	5.8
Hygienic attitude of after their needs such as toilet, cigarette, lunch break*						
Hand washing	44	100.0	54	91.5	98	95.1
Cologne	6	13.6	11	18.6	17	16.5
Disinfectant	1	2.3	12	20.3	13	12.6
Diseases in the past year						
Absent	8	18.2	17	28.8	25	24.3
Respiratory system diseases	26	59.1	31	52.5	57	55.3
COVID-19	7	15.9	10	16.9	17	16.5
Other	3	2.3	1	1.7	4	4.0
Position at work						
Business owner-partner	9	20.5	17	28.8	26	25.2
Permanent employee	28	63.6	34	57.6	62	60.2
Part time employee	7	15.9	8	13.6	15	14.6
Storage place of participant's masks						
Do not store	8	18.2	11	18.6	19	18.4
Chin	11	25.0	6	10.2	17	16.5
Arm	2	4.5	6	10.2	8	7.8
Pocket	13	29.5	21	35.6	34	33.0
Bag	6	13.6	2	3.4	8	7.8
Hanger	2	4.5	4	6.8	6	5.8
Countertop	2	4.5	9	15.3	11	10.7
Mask usage duration						
< 4 h	5	11.4	12	20.3	17	16.5
4-8 h	5	11.4	14	23.7	19	18.4
9-24 h	21	47.7	22	37.3	43	41.7
> 24 h	13	29.5	11	18.7	24	23.3
Usage of mask or other protective equipment used by others						
Yes	2	4.5	5	8.5	7	6.8
No	42	9.5	54	91.5	96	93.2
Whether people think that the use of masks protects them from diseases						
Yes	27	61.4	22	37.3	49	47.5
No	16	36.4	37	62.7	53	51.5
Indecisive	1	2.3	-	-	1	1.0
TOTAL	**44**	**100.0**	**59**	**100.0**	**103**	**100.0**

** More than one option has been ticked. X: mean; SE: standard error*

3.2. Microbiological Results

Bacterial contamination was found at different levels on all tested (103) face masks. Total aerobic mesophilic counts were found to be around 9 $\log_{10}$ CFU/mL, while yeast and mould counts were approximately 8 $\log_{10}$ CFU/mL in each age group as indicated in Figure 1. There was no statistically significant relationship between bacterial growth and gender. Coliform findings indicate a bacterial growth range between 8.41-10.56 $\log_{10}$ CFU/mL.

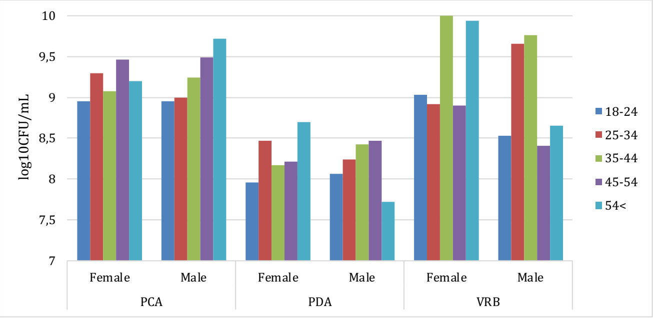

***Figure 1.** Total bacterial counts (log10 CFU/mL) within indicated age groups*

Figure 2 shows that the highest coliform counts (10.56 $\log_{10}$ CFU/mL) were seen in women aged 35 – 44 (p <.05). It is notable that the mean counts isolated from part-time employees was found to be lower than that of permanent employees and owners (p > .05). This might be due to the mask being changed more frequently.

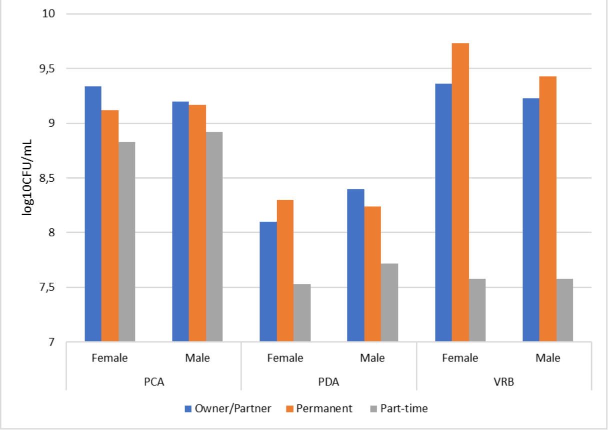

***Figure 2.** Total bacterial counts ($\log_{10}$ CFU/mL) within indicated work position*

3.2.1. Identification Results by MALDI-TOF MS

In current study, the number, ratio and habitats of microorganisms classified by MALDI-TOF MS are presented in Table 2.

Bacillus cereus was detected at the highest level with 17.86%. This was followed by *Enterobacter cloacae*, *Klebsiella oxytoca*, *Kurthia gibsonii* and *Pseudomonas aeruginosa* with rates of 14.29%, 10.72%, 7.14%, and 7.14%, respectively. All of the other classified microorganisms were detected at the level of 3.57%. When the detected species were classified according to their families, it was seen that the species belonging to *Enterobacteriaceae*, which also included pathogens, were dominant, followed by the *Bacillaceae* family, which is generally composed of ubiquitous environmental bacteria, with 25%.

Table 2. *Number and percentage of prevalence of microorganisms detected via MALDI-TOF MS in surgical mask samples of food industry employees.*

Organism identified	Number	%	Typical habitat
Firmicutes			
Bacillaceae			
Bacillus cereus	8	17.86	ubiquitous, environment, soil
Bacillus subtilis	1	3.57	ubiquitous, environment, soil, water
Lysinibacillus pakistanensis	1	3.57	ubiquitous, environment, soil, water
Planococcaceae			
Kurthia gibsonii	2	7.14	ubiquitous, intestinal tract
Enterococcaceae			
Enterococcus faecalis	1	3.57	gut, feces
Proteobacteria			
Enterobacteriaceae			
Enterobacter cloacae	4	14.29	ubiquitous, environment, soil, water, intestinal tract
Enterobacter xiangfangensis	1	3.57	ubiquitous, environment, soil, water, intestinal tract
Klebsiella oxytoca	3	10.71	ubiquitous, environment, colon, nasopharynx, skin
Klebsiella pneumoniae	1	3.57	ubiquitous, environment, mouth, skin, intestinal tract
Pseudomonadaceae			
Pseudomonas aeruginosa	2	7.14	ubiquitous, environment, soil, water, plant, nasopharynx, skin, intestinal tract
Moraxellaceae			
Acinetobacter pittii	1	3.57	environment, sewage, soil, plant, water, skin, foods
Acinetobacter radioresistens	1	3.57	environment, soil, water, skin, cotton
Erwiniaceae			
Mixta calida	1	3.57	plant, food products
Ascomycota			
Saccharomycetaceae			
Candida lusitaniae	1	3.57	skin, mouth, vaginal mucous membranes, feces
Total	28	100.0	

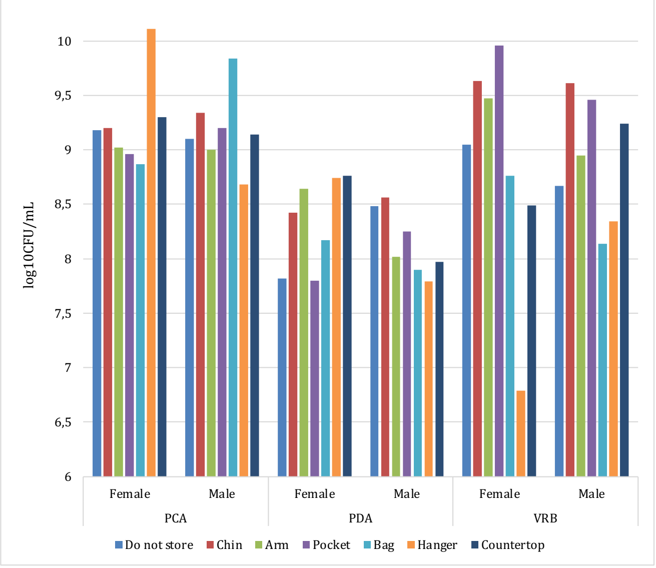

Figure 3. *Total bacterial counts (log_{10} CFU/mL) within indicated storage places*

4. DISCUSSION

When the hygienic attitudes of the people were questioned after their needs such as toilet, cigarette, and meal breaks, it was determined that all the women (n=44) and 95.1% of the men washed their hands after the specified needs (Table 1). Hand washing is extremely important for the protection and improvement of public health (23). Like this study, the Turkey hand washing research report stated that hand washing rates were high (24). It was observed that only 18.4% (n=19) of the individuals did not store the mask they used or used a new mask. The WHO has published a report as a mask use guide in the COVID-19 pandemic. Suggestions such as 'perform hand hygiene before putting on the mask, inspect the mask for tears or holes, and do not use a damaged mask, replace the mask as soon as it becomes damp with a new clean, dry mask, either discard the mask or place it in a clean plastic resalable bag where it is kept until it can be washed and cleaned, do not store the mask around the arm or wrist or pull it down to rest around the chin or neck, do not share your mask with others, discard single-use masks after each use and properly dispose of them immediately upon removal' have been developed in this report (25). 41.7% (n=43) of the individuals participating in this study stated that they used a mask for 9-24 hours, and 23.3% (n=24) used it for more than 1 day. Based on these findings, it is believed that the masks are being handled improperly, and that personnel should be trained in this regard.

Figure 2 shows that the highest coliform counts (10.56 log_{10} CFU/mL) were seen in women aged 35 – 44 ($p< .05$). It is notable that the mean counts isolated from part-time employees was found to be lower than that of permanent employees and owners ($p > .05$). This might be due to the mask being changed more frequently. WHO suggested that the masks do not store the mask around the arm or wrist or pull it down to rest around the chin or neck (25).

The storing places of the masks used by the participants in this study are shown in Table 1. The bacterial load of the masks used by the participants according to the hiding places of the masks is shown in Figure 3. Sachdev et al. (26) found similar results in their study. Accordingly, the microbiological counts of the participants in the current study on the mask storage areas have again proven the importance of WHO's recommendations.

Masks have traditionally been recognized to serve a significant role in ensuring workplace hygiene. However, it is worth debating whether the measures in place to prevent germs or viruses shed by employees can become a source of bacterial contamination. In this study, we investigated the bacterial contamination of face masks used by food industry employees. The mean bacterial counts rose with increased wearing time, although this was not statistically significant. Similar to this study, Zhiqing et al.(27) reported that the bacterial count on the surface of surgical masks increased with extended operating times. Several bacterial pathogens including *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Kurthia gibsonii* etc. were detected. According to research, germs may be produced and spread via exhaled breath, offering an elevated risk of infectious illness transmission (28). Several investigations have indicated airborne and/or droplet transmission as the primary pathways for transmitting human and animal germs such as *E. coli*, *S. aureus*, *K. pneumonia*, and *P. Aeruginosa* (29, 32).

When the detected species were classified according to their families, it was seen that the species belonging to *Enterobacteriaceae*, which also included pathogens, were dominant, followed by the *Bacillaceae* family, which is generally composed of ubiquitous environmental bacteria, with 25%. Gund et al. (33), who applied a method similar to our study methodologically, reported that 79% of the masks of dental clinic workers were contaminated, and *Staphylococcus epidermidis*, the natural habitat of which was skin, was the most common. Similarly, in a study conducted with 130 dental clinic participants, the count and genus of microorganisms in used masks were determined by classical cultural techniques, and it was stated that bacterial species was predominated by *Staphylococci* species 26.35% followed by *Pseudomonas* 17.82% and *Streptococci* 15.50%. It is seen that the natural habitats of the bacteria detected in this study are the skin and nasopharynx (26).

One of the most striking results of this research was the distribution of the species identified from the masks according to their natural habitats. It was determined that 53.16% of the species were of intestinal origin. This shows that the hygiene attitudes of the employees are quite bad, and they gave misleading answers to the questions about hygiene in the questionnaire part of the research.

5. CONCLUSION

The current study was performed to determine mask usage habits and to assess the presence of bacteria and fungi among food industry employees. Participants of this study's cross-sectional questionnaire lacked sufficient information about how to use masks properly in the workplace. Although the majority reported to replace the mask on a regular basis, the results show that the main source of contamination in the mask is the users' hands and their tendency of putting them in locations like the mouth, arm, and pocket, creating external contamination. Similarly, although the majority (95.1%) of the food industry employees participating in the study claim that they have hand washing habits, it is thought that the microbial contamination in the masks they use is due to insufficient hygiene. The fact that 71.8% of the participants suffered from infections in the last year also supports this situation. The use of masks containing high levels of germs in food facilities owing to poor cleanliness and storage conditions is regarded as a cause of aerosol formation. In the present study, long-term working conditions, especially inaccuracies in the use of masks, poor hygiene attitudes of the employees, and the fact that the employees wear the mask out of necessity rather than its protective effect suggest that is why the poor microbiological quality of the analyzed masks. The results show that since most of the masks to protect yourself from COVID-19 and prevent infecting others used by food industry employees contain bacteria of intestinal origin, serious health problems may occur both in employees using contaminated masks and in consumers because of contamination of food by masks. Finally, the results could have been affected by the fact that the data was not collected at the beginning of the pandemic and, consequently, the tendency of people to pay fewer attention to masks or protective equipment with time. Therefore, this situation may be regarded as a limited aspect of the study.

Acknowledgement: *This study was conducted in Burdur Mehmet Akif Ersoy University, Veterinary Faculty, Department of Food Hygiene and Technology, and Hatay Mustafa Kemal University Plant Health Clinic Application and Research Center*

Funding: *This study received no financial support.*

Conflicts of interest: *The authors report no conflict of interest.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Non-Interventional Clinical Research Ethics Committee of Burdur Mehmet Akif Ersoy University (2021/422)*

Peer-review: *Externally peer-reviewed.*

Author Contributions:

Research idea: AHD, MÖ

Design of the study: AHD, MÖ
Acquisition of data for the study: AHD, EBÖ, ZÇ, JR
Analysis of data for the study: MÖ
Interpretation of data for the study: AHD, JR
Drafting the manuscript: MÖ, EBÖ, ZÇ, JR
Revising it critically for important intellectual content: AHD, MÖ
Final approval of the version to be published: AHD

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How to cite this article: Dinçoğlu AH, Özgür M, Özgür EB, Çalışkan Z, Rugji J. The Used Masks of The Food Industry Employees During the Covid-19 Pandemic: Did the Mask Promote Other Diseases While Protecting from the Coronavirus? A Survey Study Supported by Microbiological Data. Clin Exp Health Sci 2024; 14: 431-438. DOI: 10.33808/clinexphealthsci.1326608