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PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 43, No. 3, p. 215–222
https://doi.org/10.4315/FPT-22-030

Face Masks as Sources of Cross-Contamination during Food Preparation

ABSTRACT

Amid the COVID-19 pandemic, mask-wearing has become a common practice in the foodservice industry to prevent the spread of respiratory diseases. Like kitchen utensils, a mask may serve as a vehicle for cross-contamination of pathogens during food handling. The objective of this study was to quantify cross-contamination between tasks of handling contaminated chicken and chopping lettuce. Chicken breasts were inoculated with a high or a low level of nonpathogenic *Escherichia coli* surrogates (ca. 6 or 4 log CFU/ml) and sliced for 1, 5, or 10 min. During slicing, duplicate, single-use medical masks were touched each minute. One mask was immediately sampled, but the second mask was used to contaminate lettuce by touching the mask each minute while chopping the lettuce for 5 min. *E. coli* were enumerated from the second mask and lettuce. Masks touched while slicing both high- and low-inoculated chicken showed significant contamination (0.8–4.9 log CFU/cm^2) after each slicing scenario of 1, 5, or 10 min ($P > 0.05$). Lettuce was significantly contaminated regardless of inoculation level (1.0–3.2 log CFU/g). Slicing time was a significant factor in some cases ($P < 0.05$), whereas inoculation level was not ($P > 0.05$). Data indicate masks can be a source of cross-contamination if not replaced appropriately.

INTRODUCTION

Cross-contamination is one of the most common errors during food preparations that can lead to foodborne illness. These incidents may occur within individuals' homes or in food establishments and may be the cause of up to one-third of foodborne illnesses *(6, 21)*. Many studies have identified specific sources of cross-contamination in both residential and commercial kitchens. The use of improperly cleaned utensils and surfaces, as well as multiuse towels, have shown cross-contamination in residential and commercial-styled kitchen scenarios *(1, 4, 11, 12, 15, 17, 19)*. Efforts have been made by university extension specialists and health agencies to educate restaurant employees about cross-contamination, such as emphasizing proper hand-washing techniques and changing tools between tasks *(2, 24)*. However, poor hand and employee hygiene is a commonly cited infraction and a source for foodborne illnesses *(8, 20)*.

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The COVID-19 pandemic has led to mask-wearing in public and particularly in restaurant and hospitality settings where there may be close human-to-human interaction. Educating the public on properly fitting a mask, whether single-use medical or reusable cloth, has been a challenge, as well as communicating best practices about mask reusability. Mask-wearers may frequently adjust the mask to fit properly over the nose, mouth, and chin when talking or performing activities. Although there is no evidence to suggest transmission of SARS-CoV-2 via fomites (hands, surfaces, foods, etc.), there have been studies to show persistence of the virus on masks for up to 21 days on inoculated personal protective equipment when soils were present, which was investigated specifically to address how long masks could be used before replacing *(13)*. Because of supply chain shortages, expense, and implications about waste and pollution, increasing the time or frequency that a single mask could be worn has been a significant topic of discussion. However, adjusting and touching a mask while handling contaminated raw and ready-to-eat (RTE) foods could lead to transmission of diseases, such as those caused by foodborne pathogens. Some information has been provided about how to reuse masks safely, such as handling the mask by the ear loops or head straps and storing in paper sacks to allow the mask to dry (for pathogen die-off), implying that there is an assumption that used masks are considered contaminated and require careful handling *(3)*.

During the COVID-19 pandemic, the authors observed a cooking class in which the chef adjusted their mask while performing food preparation demonstrations. If foodborne diseases can be transmitted because of cross-contamination of utensils or towels, it is possible that a mask could also serve as a vehicle of foodborne pathogens if not changed when contaminated. For example, touching a mask while handling or preparing raw foods followed by touching a mask while preparing RTE foods could plausibly create a cross-contamination scenario in which pathogens are transferred from raw to RTE foods. In an observational study, cross-contamination occurred up to 43 times in a single meal preparation activity *(16)*, and current efforts to mask in public settings and foodservice establishments could increase the number of or opportunities for cross-contamination if masks must be adjusted frequently.

The objectives of this project were to evaluate masks as vehicles of pathogen cross-contamination between food-handling tasks (slicing contaminated raw chicken and chopping lettuce) and determine which factors (inoculation level and slicing time or number of touches), if any, significantly affect the transfer of bacteria from inoculated chicken to masks and from contaminated masks to lettuce. To address these objectives, lab personnel sliced contaminated raw chicken while touching a medical mask at predetermined time intervals. The masks were then used as a contamination source while chopping lettuce by touching the mask at predetermined time intervals. Inoculated chicken, masks, and lettuce were sampled, and *Escherichia coli* were enumerated to determine the log CFU per gram or square centimeter from chicken to mask and mask to lettuce. The present research highlights another potential route of cross-contamination during food preparation. Food safety training professionals, as well as those who work in laboratory settings, should consider developing protocols and recommendations for employees that encourage using disposable masks and changing them between tasks.

MATERIALS AND METHODS

Design of experiments

To quantify cross-contamination via masks during food preparation, disposable medical masks were placed on a mannequin and touched at predetermined time intervals while slicing inoculated chicken breasts. Touched masks were then used as the contamination source while chopping lettuce. All samples were serially diluted and plated *onto E. coli*/Coliform (EC/C) Petrifilm (3M, St. Paul, MN). The project flow is depicted in *Fig. 1*.

Preparation of nonpathogenic surrogate panel of *E. coli*

Five *E. coli* strains (panel MP-26 [ATCC BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431], Manassas, VA) were maintained at −80°C on protectant beads (Microbank, Pro-Lab Diagnostics, Ontario, Canada) in tryptic soy broth (TSB) with glycerol (Difco, BD, Franklin Lakes, NJ). A single bead for each strain was transferred into 10 ml of TSB and incubated at 37°C for 24 h for an overnight culture. For each replication, strains were streaked individually onto tryptic soy agar (Difco) and incubated at 37°C for 24 h. Single colonies were transferred into 10 ml of TSB for overnight incubation of 37°C for 24 h. Following incubation, each strain was centrifuged (9,000 rpm [8,000 × *g*], 10 min) and washed with 0.1% peptone twice, after which 5 ml from each strain were combined to create a 25-ml cocktail (ca. 9.2 ± 0.1 log CFU/ml, counted with a Petroff-Hauser counter). From the high-inoculation cocktail, 0.25 ml was used to create a low-inoculation cocktail (ca. 6.8 ± 0.4 log CFU/ml). Each strain in the surrogate panel, as well as the high- and low-inoculation cocktails, was serially diluted in 0.1% peptone and plated on EC/C Petrifilm. The panel of nonpathogenic *E. coli* was used because it has been shown to be representative of several *Enterobacteriaceae* on meat products.

Preparation and inoculation of chicken breasts with *E. coli*

Two noninoculated chicken breasts were sampled with a sponge to ensure no EC/C was detected on the surface (EZ-Reach polyurethane sponge sampler, World Bioproducts, Libertyville, IL). Sponges were diluted with 0.1% peptone and serially diluted before plating on EC/C Petrifilm, which was used for convenience and efficiency. No EC/C was

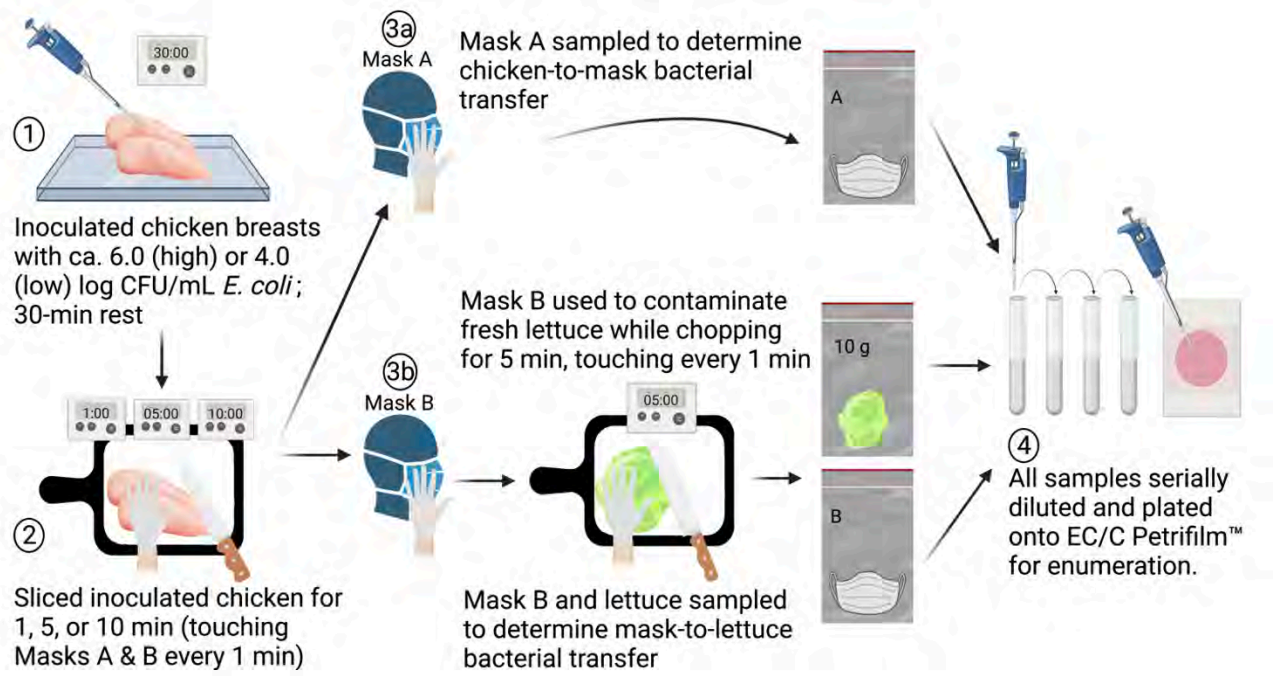

Figure 1. Project flow and design of experiment to show *E. coli* transfer from chicken to masks and from masks to lettuce.

detected. Thirty-six chicken breasts were surface-inoculated with 0.25 ml of either the high- or the low-inoculation cocktail (18 of each kind), spread and distributed with a sterile L-spreader, and allowed to rest at ca. 6°C for 15 min. The second side was inoculated in the same manner. After the 30-min attachment period, the surface of two chicken breasts was sponge-sampled (10 cm^2) to confirm consistent inoculation for both high- and low-inoculated samples (5.9 ± 0.2 and 3.8 ± 0.4 log CFU/cm^2). The researchers aimed to create a 2-log CFU difference between high and low inoculation while still achieving levels that could demonstrate significant transfer or contamination.

Contamination and sampling of masks

Two sterile disposable medical masks (Anqing Xinhui Sanitary Products Co., Anhui Province, China) were aseptically placed on mannequin heads for each experiment in which inoculated chicken (with either a high or a low level) was sliced (ca. 1-in pieces) for 1, 5, or 10 min while touching the two masks every minute. The order of experiments was randomly predetermined. Masks were touched in a consistent manner by grabbing the front of the mask (90-cm^2 area) and repositioning it onto the nose of the mannequin. After slicing, one mask was aseptically removed from a mannequin and placed into a sterile sample bag (Whirl-Pak, Nasco, Fort Atkinson, WI) with 90 ml of 0.1% peptone, stomached for 30 s, and plated on EC/C Petrifilm.

Contamination and sampling of lettuce

Fresh gloves, knives, and cutting boards were used to chop lettuce for 5 min (iceberg lettuce, washed with core and outer leaves removed, ca. 250 g), touching the second contaminated mask before chopping and every minute throughout the chopping. After the chopping period, the second mask was aseptically removed and sampled as previously described for the first mask. Chopped lettuce was weighed (10 g) and added to a sterile sample bag with 90 ml of 0.1% peptone. The sample bag was stomached for 30 s, serially plated on EC/C Petrifilm, and incubated at 37°C for 24 h.

Statistical analysis

Experiments were designed as randomized complete blocks with a two-factor factorial. The first factor was the inoculation level of the chicken (low or high), and the second factor was touching time (1, 5, or 10 min). The individual performing the cross-contamination was considered a block because of potential variation across individuals. Three independent replicates of the experiment were performed for each combination of high vs. low inoculation and touching time and were analyzed using the following model:

$$Yijk = \text{block}_k + \text{inoculation}_i + \text{mask-touching}_j + \varepsilon ijk,$$
$$i = 1, 2;\ j = 1, 2, 3;\ \text{and}\ k = 1, 2, 3$$
$$\text{where}\ \varepsilon ijk \sim N(0,1)$$

TABLE 1. Average log CFU per gram or square centimeter (±SD) of *E. coli* recovered from inoculated chicken, mask A and B contaminated from chicken, and lettuce contaminated from mask B

Inoculation level	Slicing time (min)[a]	Log CFU/g or log CFU/cm² ± SD			
		Chicken	Mask A[b]	Mask B[c]	Lettuce
High	1	5.9 ± 0.2	3.2 ± 0.8	2.3 ± 1.1	2.0 ± 0.8
	5		4.8 ± 0.2	4.1 ± 0.5	3.2 ± 0.2
	10		4.6 ± 0.5	3.4 ± 0.7	2.8 ± 0.5
Low	1	3.8 ± 0.4	1.4 ± 0.3	0.9 ± 0.2	1.0 ± 0.0
	5		2.6 ± 0.2	1.5 ± 0.4	1.1 ± 0.2
	10		2.8 ± 0.4	2.1 ± 0.6	1.4 ± 0.5

[a]Slicing time refers to the number of minutes the chicken was sliced while touching masks once per minute. All lettuce was chopped for 5 min while touching the contaminated mask once per minute.

[b]Mask A was sampled immediately after chicken was sliced.

[c]Mask B was sampled following chopping lettuce, in which it was used as the contamination source.

The interaction term (inoculation × mask-touching)$_{ij}$ was excluded because no effect was detected. The response ($Yijk$) was investigated in two parts: 1) transfer from chicken to mask and 2) transfer from mask to lettuce. Rank statistics were used to evaluate the ratio of the transferred bacteria from chicken to mask because the levels were on vastly different scales. Use of ranks allowed better understanding of data values with different scales, was free of assumptions (such as the assumption of normality in a statistical model), and is invariant to the log transformations. Analysis of variance was used for subsequent analyses. All data analyses were conducted in R Studio (R version 3.6.3) *(18)*.

RESULTS

In studies that characterize cross-contamination, data may be log-transformed and compared, or the percent transfer may be calculated *(11, 12)*. Although this transformation can produce normalized data, it is not always a transformation that yields normalized data that can be meaningfully compared. The present study chose to log-transform CFU per gram or square centimeter, calculate a transfer ratio, and conduct rank statistics to evaluate significance after normalization. The rank statistics allowed meaningful comparison regardless of the difference in inoculation level so that conclusions would not be drawn simply based on the magnitude of difference between high and low inoculation. *Table 1* shows the log CFU per gram or square centimeter of *E. coli* recovered from each chicken, mask, and lettuce sample, and *Fig. 2* shows the general progression of transfer (recovery of *E. coli*) for each contamination and slicing scenario in the form of a scatterplot. Transfer of *E. coli* was observed from inoculated chicken to masks and from contaminated masks to lettuce. The difference in inoculation level did not affect the ranking of bacterial transfer. However, the number of touches between inoculated chicken and mask did result in greater bacterial transfer. The ranked statistical approach allowed normalization of data and highlighted that contaminating a mask 10 vs. 1 time contributed to significant transfer of *E. coli* but that the difference between touching a mask 5 vs. 10 times was not significant. Furthermore, the number of times the mask was touched during chicken slicing did not proportionally affect bacterial transfer from the mask to the lettuce. Mean and standard deviation (SD) of bacterial transfers are reported in *Table 1*.

Transfer of *E. coli* from inoculated chicken to masks

Overall, the slicing time and subsequent number of touches to the mask while slicing the inoculated chicken significantly affected *E. coli* transfer. Evident in the normalized ranking statistics, the more times a mask was contaminated, the greater the bacterial transfer ($P < 0.05$). In the case of slicing high-inoculated chicken breasts, the transfer to the mask when touched every minute for 1, 5, and 10 min was 3.2, 4.8, and 4.6 log CFU/cm², respectively. In the case of low-inoculated chicken breasts, touching masks every minute for 1, 5, and 10 min resulted in recovery of 1.4, 2.6, and 2.8 log CFU/cm², respectively. Some touching time intervals (1, 5, and 10 min) had significant effects on transfer of *E. coli*. Significant differences were found between slicing times of 1–5 min ($P < 0.05$) and 1–10 min ($P < 0.05$), with

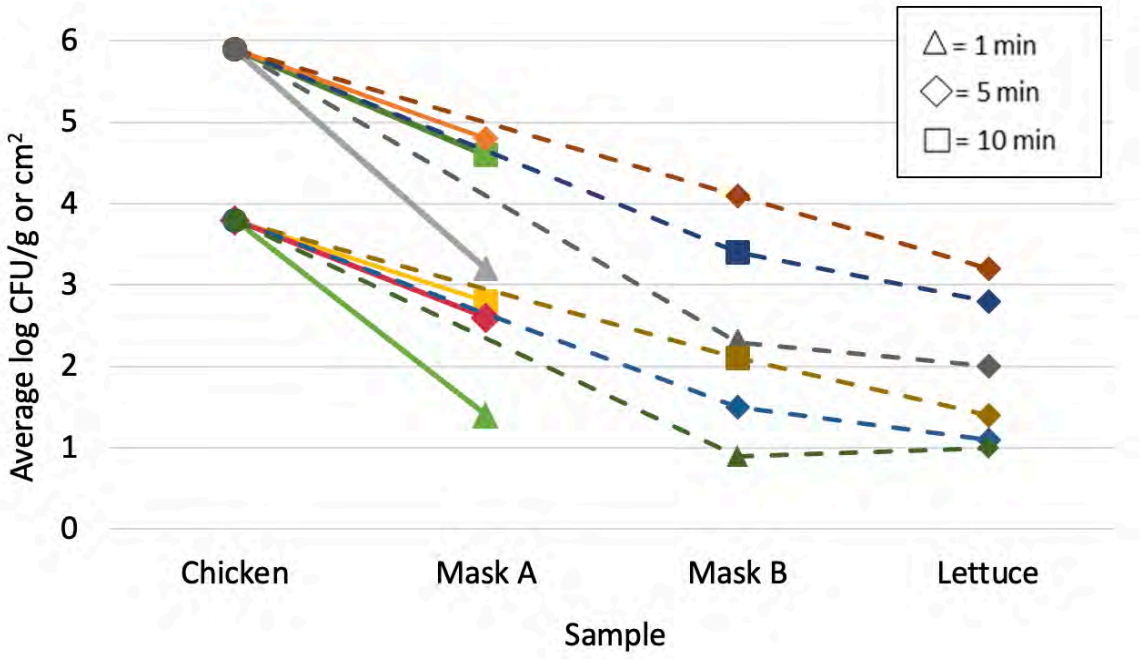

Figure 2. Sequential transfer of *E. coli* from chicken to mask and from mask to lettuce. Log CFU per gram or square centimeter of *E. coli* on chicken, mask, and lettuce samples. Connecting lines indicate sequential transfer from chicken to mask A (solid) and from chicken to mask B to lettuce (dashed). Different markers correspond to chicken slicing time.

no significant differences in transfer of *E. coli* for the 5- and 10-min slicing intervals ($P > 0.05$).

Effect of inoculation level on bacterial transfer

Although inoculation level affected how much *E. coli* was transferred to the mask from the sliced chicken (ca. 2-log CFU/cm² difference), differences after ranking normalization of transfer rates were statistically insignificant ($P > 0.05$). *Fig. 3* depicts boxplots of transferred *E. coli* from low- and high-inoculated chicken breast to masks, and significant overlap can be seen. Furthermore, inoculation level had no effect on the transfer rate (rank) of *E. coli* from mask B to lettuce ($P > 0.05$). A boxplot of *E. coli* transfer from mask B to lettuce (not shown) also revealed significant overlap and bore an almost identical resemblance to *Fig. 3*. Had inoculation level significantly affected the transfer, the boxplots would not have shown overlap with ranks; instead, groupings would have been observed in the rank statistics based on inoculation level.

Transfer of *E. coli* from contaminated masks to lettuce

For all contamination scenarios, the lettuce was chopped for 5 min and the contaminated mask was touched every minute, such that the lettuce samples were contaminated the same number of times regardless of the chicken inoculation level, amount of time chicken was sliced, and number of times the mask was touched. Results showed that neither slicing time nor inoculation level (noted earlier) significantly affected *E. coli* transfer from masks to lettuce *(Fig. 4)*. The transfer of *E. coli* from mask B to lettuce ranged from 2.0 to 3.2 log CFU/g (contaminated from high-inoculated chicken) and 1.0 to 1.4 log CFU/g (contaminated from low-inoculated chicken). As with the initial mask contamination, larger transfers of *E. coli* occurred for the high-inoculated samples, although this did not affect the ratio or rank ($P > 0.05$). Masks that were only touched 1 time while slicing chicken still resulted in a significant transfer of *E. coli* to the lettuce that was comparable to masks that were touched 5 or 10 times while slicing chicken. Ultimately, the slicing time and the respective number of times the masks were contaminated did not affect the *E. coli* transfer rate from mask B to lettuce ($P > 0.05$).

DISCUSSION

Amid the COVID-19 pandemic, heightened interest has been shown by consumers and the media for food safety. Hundreds of videos and other types of messaging were produced in the United States and Canada to discuss food handling practices. Although SARS-CoV-2 is not a foodborne pathogen, many consumers and health professionals were concerned with contamination scenarios in which the virus could spread through food as a fomite. Consumers noted that food handling and preparation related to coronavirus risk were of the highest concerns for food safety *(10)*. According to a survey by Fanelli *(5)*, 70% of Italian consumers noted that food safety was a priority during a health emergency,

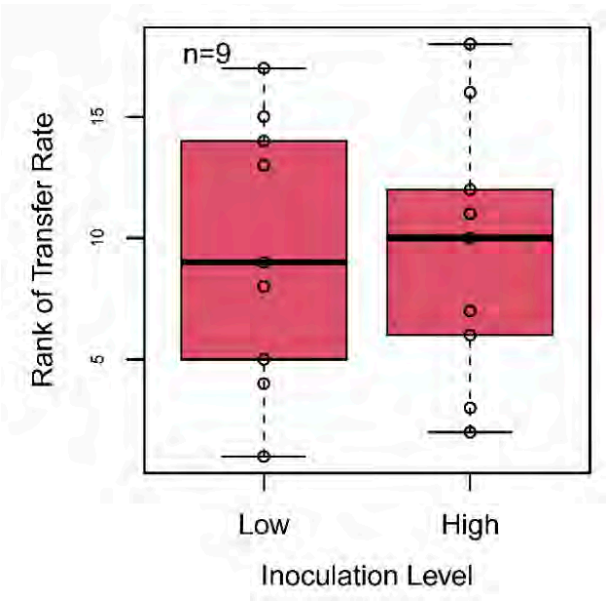

Figure 3. Boxplot of transfer rate rankings from chicken to masks based on inoculation level (low vs. high).

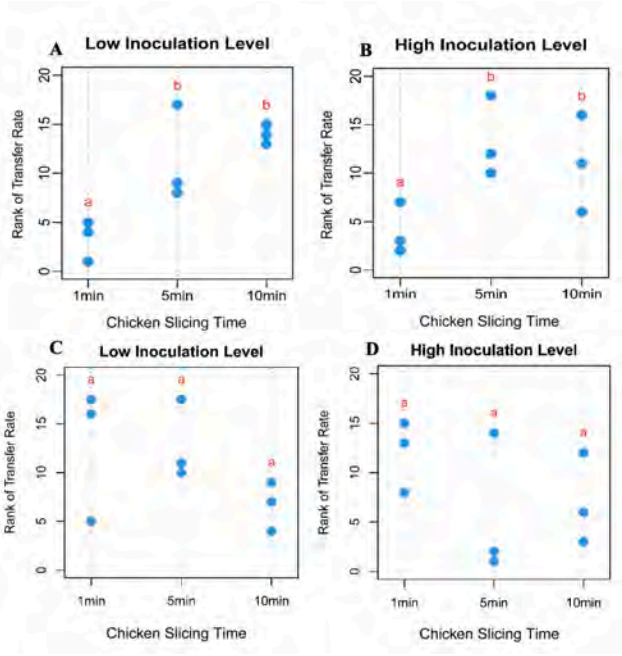

Figure 4. Ranking of transfer rates grouped by chicken slicing times. Letters of significance show significant differences in slicing times (number of times the mask was touched) for chicken to mask A (A and B) and for mask B to lettuce (C and D). Mask B to lettuce resulted in no significant differences in the rank of transfer rates based on initial chicken slicing time.

highlighting the heightened interest. It is vital that safe food handling practices be communicated to consumers and food handlers during health emergencies, particularly if consumer behavior changes significantly, such as wearing masks.

Cross-contamination is one of the most common problematic food handling practices in residential and commercial foodservice establishments and has been investigated by many studies. Some studies have noted gloves or hands as vehicles for bacterial transfer and characterized or quantified contamination on surfaces and utensils. These studies showed that cross-contamination and bacterial transfer easily occur when food handlers do not appropriately change gloves, towels, or utensils between some food preparation tasks, but the degree to which this occurs is affected by surface, bacteria, or material *(7)*. Cross-contamination or breaches in aseptic practices is of great interest outside the food industry, such as in healthcare and dental industries. In a study to determine whether bacteria could be transferred from masks to gloved hands, masks were contaminated during aerosol-causing dental procedures *(9)*. Researchers recovered contaminants (*Streptococci* spp. and *Staphylococci* spp.) from masks, as well as gloves that touched the contaminated masks. Most masks worn by the public throughout the pandemic in the United States were disposable medical masks or reusable cloth masks that are not designed to have a tight fit, requiring frequent manual adjustment to cover the nose, mouth, and chin. It was unknown whether or how bacteria transfer to and from these masks in the context of food preparation. Therefore, the authors of the present study were intrigued by the potential of cross-contamination via disposable medical masks commonly worn during the COVID-19 pandemic while handling raw and RTE foods *(Fig. 1)*. The results highlighted that food handlers, when wearing a mask to prevent disease transmission, must change the mask between tasks or wear a properly fitting mask to prevent the need to adjust it periodically.

Because initial inoculation level (high vs. low) did not have a significant impact, the results indicate that even if the natural contamination level is low, cross-contamination may still occur and lead to foodborne illness. Other studies have shown or suspected impacts of inoculation level, calling into question whether a high inoculation level should be used in studies such as these *(11)*. For example, Montville and Schaffner *(14)* found that inoculum size or concentration had a significant effect on the percent transfer rate of bacteria from surfaces (cutting boards, hands, gloves, and spigot) to foods (chicken and lettuce) and from foods to surfaces. They stated that studies determining cross-contamination might be affected significantly enough by the inoculum size to confound conclusions. Others have pointed out that bacterial transfer studies that examine stepwise or cumulative transfer run the risk of overestimating *(22)*. These points, as well as the unlikelihood of natural contamination as high as 5 log CFU/g pathogen in these scenarios, spotlight the criticism of using only high inoculation levels in laboratory studies. Therefore, two inoculation levels were chosen for the present study to see whether trends in bacterial transfer

rates were significantly affected by the initial inoculation concentration. Because the experimental design involved two subsequent transfers and a limit of detection of 1 log CFU/g or 1 log CFU/cm^2, the authors determined that meaningful results might be lost or unobserved if the initial bacterial concentration was too low. The twofold difference between high and low inoculation yielded insignificantly different results. Therefore, the authors presume that an even lower inoculation level of less than 4 log CFU/ml would have yielded similar results.

Other studies have shown significant cross-contamination of microorganisms while performing food preparation tasks that involve transfer between different matrices and via fomites (hands, surfaces, foods, etc.). Transfer rates via fomites are not consistent across all studies and scenarios for various reasons, such as material, task, and microorganism *(11)*. One study showed a 1-log difference between side-by-side *Campylobacter jejuni* and *Lactobacillus casei* transfer from inoculated chicken to produce *(22)*. This difference may result from attachment or stress resistance differences. Although single-use medical masks may not be made of material that is conducive to the growth or survival of microorganisms, nonpathogenic *E. coli* proved to successfully transfer via masks. It is likely that other *Enterobacteriaceae* could transfer from raw to RTE foods. Other variability in bacterial transfer rates have been noted due to uncontrollable differences in how participants complete a cross-contaminating task. For example, the transfer rate from a contaminated faucet spigot to clean hands ranged from 0.021 to 72.4%, which researchers surmised might have resulted from the differences in how much participants dried their hands after touching the wet spigot *(4)*. Conducting experiments that replicate at-home or commercial kitchen common practices provides realistic results and conclusions, but the variability may be higher *(22)*.

The observed bacterial transfer to lettuce may have been affected by the produce matrix (high moisture) and the time (5 min) spent chopping the lettuce. Verhoeff-Bakkenes et al. *(23)* demonstrated fairly high and consistent transfer rates of bacteria from chicken to a fruit salad (17–38%), whereas Jensen et al. *(11)* demonstrated slightly lower rates of transfer. The difference, they pointed out, may have been in the types of produce being handled, as well as differences in preparation and contact time *(11)*. For example, cutting and preparing an entire fruit salad involved more handling *(23)* and opportunities for transfer, whereas briefly handling freshly cut pieces *(11)* limited the contact time significantly. A reasonable consideration for the high amount of bacterial transfer from mask to lettuce in the present research is that the lettuce held and released a significant amount of moisture and was handled for 5 min, which resulted in a great deal of mixing of the product on the cutting board.

There were some limitations to the experimental design of this study and unanswered questions that are notable. For example, the researchers did not investigate the effects of drying on the masks. Although the individual slicing of inoculated chicken transitioned immediately to chopping lettuce, the changeover between the two tasks varied, at times, by 3–5 min. This may have resulted in some drying of the mask and subsequent reduction of *E. coli* populations. However, because *E. coli* was seen to transfer to the lettuce consistently regardless of other experimental factors, the drying effects are assumed to have been minimal.

CONCLUSIONS

Data presented in this study demonstrate that masks or face coverings could serve as vehicles for foodborne pathogens, because contamination of *E. coli* from chicken to lettuce occurred when masks were touched during and between food preparation tasks. Regardless of chicken inoculation level or how many times the masks were touched during slicing, *E. coli* was transferred to lettuce when the mask was touched while chopping. However, masks that were touched more often (longer slicing time) resulted in higher amounts of transfer in some cases (e.g., 4.6 vs. 3.2 log CFU/cm^2 recovered from masks touched over 10 min vs. 1 min while slicing chicken). The presented data strongly suggest that masks can serve as vehicles for pathogens, which is notable for the foodservice industry in terms of worker hygiene. This should also be of interest to laboratory researchers. During the pandemic, researchers in laboratories were encouraged to wear a mask when working with others. Touching and adjusting a mask to cover the nose, mouth, and chin should be considered a breach in aseptic technique, warranting the changing of the mask.

Although masks have been shown help prevent the spread of some respiratory diseases, great care must be taken to prevent cross-contamination by changing masks between foodservice tasks. Because mask-wearing has continued throughout the COVID-19 pandemic and will continue in the future to prevent the spread of other communicable diseases, foodservice establishments and food safety communicators should consider incorporating this information into training about worker hygiene.

ACKNOWLEDGMENTS

Funding for this research was provided by the Arkansas Agricultural Experiment Station through the University of Arkansas System's Division of Agriculture and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture. The funding organizations did not contribute to the design of the study or the resulting manuscript.

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