Bacterial Dispersion Associated With Various Patient Face Mask Designs During Simulated Intravitreal Injections

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• PURPOSE: We sought to investigate bacterial dispersion with patient face mask use during simulated intravitreal injections.
• DESIGN: Prospective cross-sectional study.
• METHODS: Fifteen healthy subjects were recruited for this single-center study. Each participant was instructed not to speak for 2 minutes, simulating a "no-talking" policy, while in an ophthalmic examination chair with an blood agar plate secured to the forehead and wearing various face masks (no mask, loose fitting surgical mask, tight-fitting surgical mask without tape, tight-fitting surgical mask with adhesive tape securing the superior portion of the mask, N95 mask, and cloth mask). Each scenario was then repeated while reading a 2-minute script, simulating a talking patient. The primary outcome measures were the number of colony-forming units (CFUs) and microbial species.
• RESULTS: During the "no-talking" scenario, subjects wearing a tight-fitting surgical mask with tape developed fewer CFUs compared with subjects wearing the same mask without tape (difference 0.93 CFUs [95% confidence interval 0.32-1.55]; *P* = .003). During the speech scenarios, subjects wearing a tight-fitting surgical mask with tape had significantly fewer CFUs compared with subjects without a face mask (difference 1.07 CFUs; *P* = .001), subjects with a loose face mask (difference 0.67 CFUs; *P* = .034), and subjects with a tight face mask without tape (difference 1.13 CFUs; *P* < .001). There was no difference between those with a tight-fitting surgical mask with tape and an N95 mask in the "no-talking" (*P* > .99) and "speech" (*P* = .831) scenarios. No oral flora were isolated in "no-talking" scenarios but were isolated in 8 of 75 (11%) cultures in speech scenarios (*P* = .02).
• CONCLUSION: The addition of tape to the superior portion of a patient's face mask reduced bacterial dispersion during simulated intravitreal injections and had no difference in bacterial dispersion compared with wearing N95 masks. (Am J Ophthalmol 2021;223:178–183. © 2020 Elsevier Inc. All rights reserved.)

SINCE THE INTRODUCTION OF INTRAVITREAL ANTI-vascular endothelial growth factor (anti-VEGF) therapy, intravitreal injections have become one of the most commonly performed procedures in all of medicine.[1] Although these medications have excellent safety profiles, acute bacterial endophthalmitis remains an uncommon but visually devastating complication.[2] Multiple studies have evaluated potential risk factors associated with postinjection endophthalmitis.[3–7] In particular, previous studies have established that the dispersion of oral flora may be reduced by minimizing speaking during the procedure and thereby reduce the incidence of oral flora–associated endophthalmitis.[7]

Previous experimental investigations involving simulated intravitreal injections suggest that face mask use by physicians may reduce bacterial dispersion associated with speech.[8,9] However, it is unknown how patient face mask use may affect bacterial dispersion. These findings are of particular importance given that during the COVID-19 pandemic universal precautions have been established for patients and physicians to wear face masks in order to decrease potential exposure to coronavirus through respiratory secretions.[10,11]

However, there is concern that face mask use by patients during an intravitreal injection may result in increased bacterial dispersion toward the eye. Previous studies have suggested that different face mask designs may result in upward or downward bacterial dispersion.[12,13] Furthermore, in response to the critical shortage of medical face masks resulting from the COVID-19 pandemic, many patients may be wearing homemade cloth masks as recommended by the U.S. Centers for Disease Control and Prevention.[14] It is unknown how these types of face masks affect the degree of bacterial dispersion. The purpose of this study is to investigate the amount of bacterial dispersion associated with various patient face mask designs during simulated intravitreal injections.

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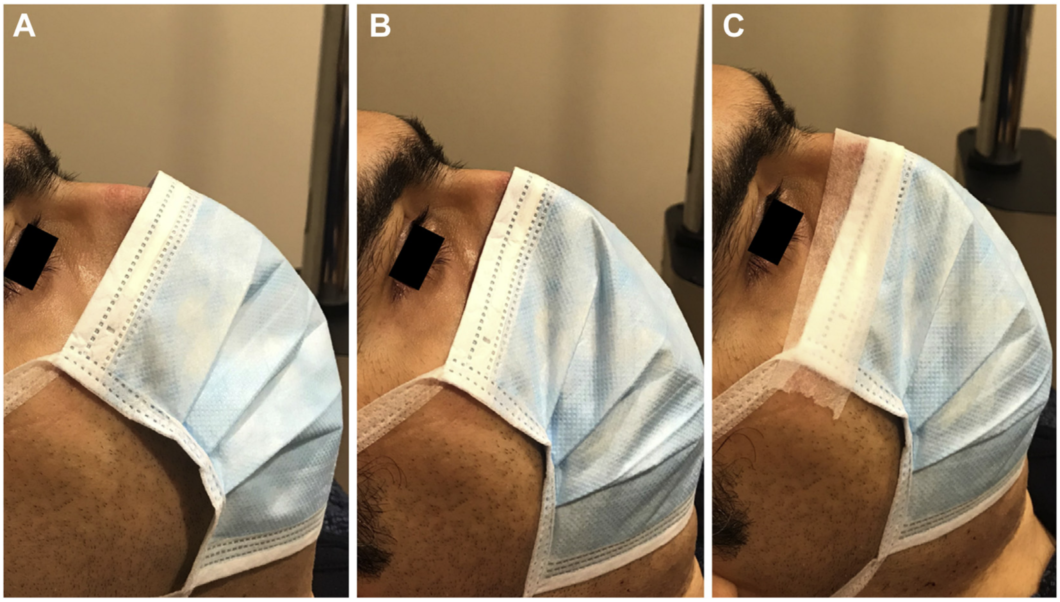

FIGURE 1. Design of the surgical face masks used during the simulated intravitreal injections. A. Loose-fitting surgical face mask that does not have the nosepiece conforming to the contour of the face and has large openings along the sides of the mask. B. Tight-fitting surgical face mask with the nosepiece conforming to the entire contour of the face. C. Tight-fitting surgical face mask with the enclosed nosepiece conforming to the entire contour of the face and adhesive tape securing the entire top portion of the face mask.

METHODS

• **OVERVIEW:** This prospective, cross-sectional, single-center study was conducted in accordance with the tenets of the Declaration of Helsinki and conformed to the Health Insurance Portability and Accountability Act. The protocol was prospectively approved by Wills Eye Hospital institutional review board, and all participants provided written informed consent.

• **STUDY PARTICIPANTS:** Fifteen healthy subjects were recruited to participate in the study as previously described.[8] Inclusion criteria included subjects ≥18 years of age with the ability to read a standardized script for 2 minutes. All subjects had previously received fit-testing for N95 face masks based on the U.S. Occupational Safety and Health Administration guidelines. Exclusion criteria included any subject with a history of upper respiratory symptoms, fever, cough, or chills within the past 2 weeks.

• **STUDY DESIGN:** Each subject was seated in an ophthalmologic examination chair in a standard examination lane at an outpatient ophthalmology office. The examination chair was reclined until the volunteer's face was approximately 45° from to the ground. A standardized 100-mm circular blood agar plate (BD BBL TSA II 5% sheep blood; Becton, Dickinson and Co, Franklin Lakes, New Jersey, USA) was then secured on the subject's forehead. Twelve scenarios were then tested. Six scenarios simulated a "no talking" policy in which the subjects were instructed to sit in silence for 2 minutes while breathing with their mouth closed. After completing the no talking scenarios, subjects were instructed to read a standardized script for 2 minutes for each of the 6 scenarios. For both the no talking and speaking scenarios, the face mask conditions included the following: 1) wearing no face mask; 2) wearing a loose-fitting surgical face mask (Halyard tie-on surgical mask; Halyard, Alpharetta, Georgia, USA; Figure 1, A); 3) wearing a tight-fitting surgical face mask (Halyard tie-on surgical mask; Figure 1, B) with the enclosed nosepiece conforming to the contour of the face; 4) wearing a tight-fitting surgical face mask (Halyard tie-on surgical mask; Figure 1, C) with the enclosed nosepiece conforming to the contour of the face and adhesive tape (Medical Tape McKesson Paper; McKesson Medical-Surgical, Irving, Texas, USA) securing the entire top portion of the face mask; 5) wearing an N95 face mask (3M Particulate Respirator 8210; 3M, St Paul, Minnesota, USA); and 6) wearing a cloth face mask (Amazon Reusable Cotton Face Mask B0891XJV1K; Amazon, Seattle, Washington, USA).

All blood agar plates were sealed and taken to Jefferson Clinical Microbiology Laboratory at Thomas Jefferson University where they were incubated for 72 hours at 37 C in a 5% carbon dioxide–rich environment. The number of bacterial colonies per plate was counted, and bacteria were identified using standard laboratory techniques by microbiologists who were masked to the plate collection

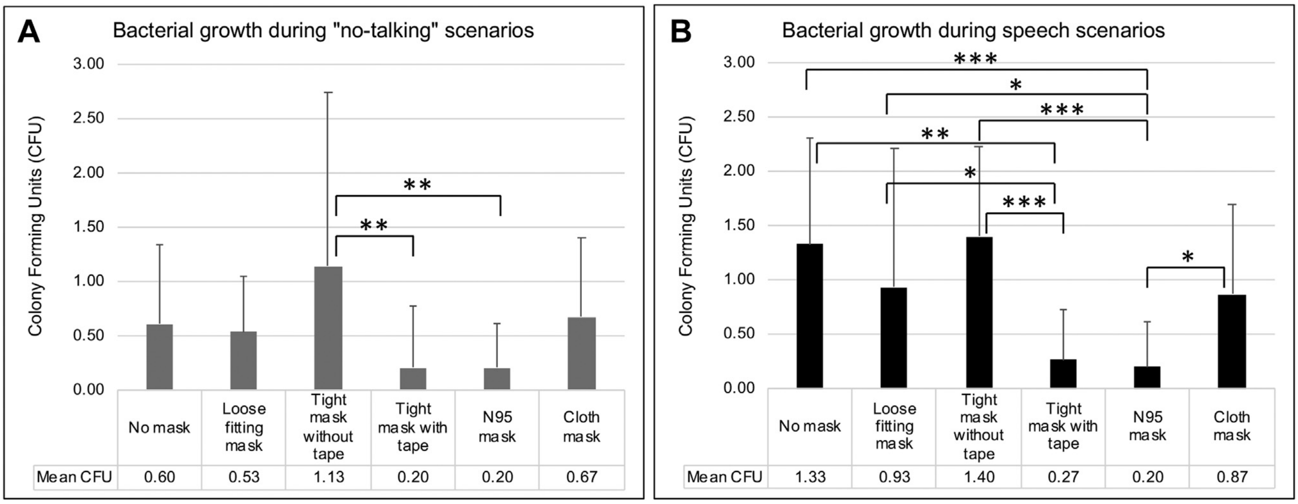

FIGURE 2. Mean bacterial growth based on colony forming units (CFUs) under various face mask conditions. A. No talking scenarios in which subjects were instructed to sit in silence for 2 minutes. B. Speech scenarios in which subjects were instructed to read a script for 2 minutes. Error bars represent standard deviations. *$P < .05$, **$P < .01$, and ***$P < .001$.

sequence. No organisms were excluded from analysis, and a culture was oral flora–associated when Enterococcus or Streptococcus species was grown on culture.

• **STATISTICAL ANALYSIS:** All data were analyzed using SPSS software (version 25; IBM Corp, Armonk, New York, USA).

The mean difference of colony-forming units (CFUs) and 95% confidence intervals (CIs) among the groups were analyzed using analysis of variance with adjustment for multiple comparisons using a Bonferroni correction. For categorical variables, significant differences between groups were analyzed using the Pearson χ^2 or Fisher exact tests. Pairwise comparisons between the no talking scenario and speech scenarios were performed using a paired Student *t* test with adjustment for unequal variances. Statistical significance was considered to be a 2-sided *P* value $< .05$.

RESULTS

FIFTEEN SUBJECTS WERE RECRUITED FOR THE STUDY, AND A total of 180 blood agar plates were successfully incubated. Overall, the mean ± standard deviation (SD) (range) CFU was 0.56 ± 0.88 (0-4) in the no talking scenarios compared with 0.83 ± 0.95 (0-6) in the speech scenarios ($P = .044$).

• **BACTERIAL DISPERSION DURING NO TALKING CONDITIONS:** Figure 2, A shows the CFUs under the no talking scenario among the different face mask conditions. During the no talking scenario, subjects with tight-fitting face mask without tape grew the most colonies with a total of 17 CFUs or a mean ± SD of 1.13 ± 1.60 per subject. In contrast, during the no talking scenario, subjects with a tight-fitting face mask with adhesive tape or those with an N95 mask both grew the fewest number of colonies with a total of 3 CFUs or a mean ± SD of 0.20 ± 0.56 and 0.20 ± 40 per subject, respectively.

Notably, during the no talking scenario, subjects wearing an N95 mask grew fewer CFUs compared with subjects wearing a tight-fitting face mask without tape (mean difference 0.93 [95% CI 0.32-1.55]; $P = .003$). In addition, during the no talking scenario, subjects wearing a tight-fitting face mask with tape grew fewer CFUs compared with subjects wearing a tight-fitting face mask without tape (mean difference 0.93 [95% CI 0.32-1.55]; $P = .003$). However, during the no talking scenario, there was no difference between subjects wearing the tight-fitting face mask with tape and subjects wearing an N95 mask (difference 0 [95% CI −0.62 to 0.62]; $P > .99$).

During the no talking scenario, there was no significant difference in mean CFUs between the no face mask and the loose-fitting face mask group ($P = .831$), between the no face mask and tight-fitting face mask groups ($P = .089$), between the no face mask and tight-fitting face mask with adhesive tape groups ($P = .201$), between the no face mask and N95 face mask groups ($P = .201$), and between the no face mask and cloth face mask groups ($P = .831$).

• **BACTERIAL DISPERSION DURING SPEECH CONDITIONS:** Figure 2, B shows the mean CFUs under the speech scenario among the different face mask conditions. During the speech scenarios, subjects wearing a tight-fitting face mask without tape grew the most colonies with a total of

TABLE. Mean Bacterial Growth with Various Face Mask Designs

Face Mask Condition	Mean ± SD CFUs During "No Talking" Scenarios	Mean ± SD CFUs During Speech Scenarios	Mean Difference (95% CI)	*P* Value
No mask	0.60 ± 0.74	1.33 ± 0.98	0.73 (0.12-1.35)	.02[a]
Loose-fitting face mask	0.53 ± 0.52	0.93 ± 1.28	0.40 (−0.22 to 1.02)	.20
Tight-fitting face mask without tape	1.13 ± 1.60	1.40 ± 0.83	0.27 (−0.35 to 0.88)	.39
Tight-fitting face mask with tape	0.20 ± 0.56	0.27 ± 0.46	0.07 (−0.55 to 0.68)	.83
N95 face mask	0.20 ± 0.41	0.20 ± 0.40	0 (−0.62 to 0.62)	>.99
Cloth face mask	0.67 ± 0.72	0.87 ± 0.83	0.20 (−0.42 to 0.82)	.52

CFU = colony forming unit; CI = confidence interval; SD = standard deviation.
[a]Statistically significant.

21 CFUs or a mean ± SD of 1.4 ± 0.82 per subject. Subjects wearing an N95 mask grew the fewest colonies with a total of 3 CFUs or a mean ± SD of 0.2 ± 0.40 per subject.

Subjects wearing a tight-fitting face mask with adhesive tape during the speech scenario grew significantly fewer CFUs compared with the following groups: 1) subjects with no face mask (mean difference 1.07 [95% CI 0.45-1.68]; $P = .001$); 2) subjects with a loose face mask (mean difference 0.67 [95% CI 0.05-1.28]; $P = .034$); and 3) subjects with a tight face mask without tape (mean difference 1.13 [95% CI 0.52-1.75]; $P < .001$).

Subjects wearing an N95 face mask during the speech scenario grew significantly fewer CFUs compared with the following groups: 1) subjects with no face mask (mean difference 1.13 [95% CI 0.52-1.75]; $P < .001$); 2) subjects with a loose face mask (mean difference 0.73 [95% CI 0.12-1.35]; $P = .02$); 3) subjects with a tight-fitting face mask without tape (mean difference 1.20 [95% CI 0.59-1.82]; $P < .001$); and 4) subjects with a cloth face mask (mean difference 0.67 [95% CI 0.05-1.28]; $P = .034$). During the speech scenario, there was no difference between subjects wearing the tight-fitting face mask with tape and subjects wearing an N95 mask (difference 0.07 [95% CI −0.55 to 0.68]; $P = .831$).

• **BACTERIAL DISPERSION BETWEEN THE SPEECH AND NO TALKING CONDITIONS:** Table summarizes the mean ± SD CFUs for each face mask design during the no talking and speech scenarios. In the no face mask scenario, subjects had significantly more mean CFUs during the speech scenario when compared with the no talking scenario (mean difference 0.73 [95% CI 0.12-1.35]; $P = .020$). There was no difference in mean CFUs between the no talking and the speech scenarios with a loose-fitting face mask ($P = .201$), a tight-fitting face mask without tape ($P = .393$), a tight-fitting face mask with adhesive tape ($P = .831$), an N95 face mask ($P > 0.99$), or a cloth face mask ($P = .521$).

• **MICROBIAL SPECTRUM:** A total of 125 CFUs were isolated with 50 isolated during the no talking scenario and 75 isolated in the speech scenario. Of the 50 CFUs in the no talking group, 0 of 50 (0%) were from oral flora, whereas 8 of 75 (11%) CFUs in the speech group were from oral flora ($P = .02$). The most common organisms isolated in the no talking scenario were Staphylococcal species (32/50, 64%).

In the speech scenarios, the most common organism isolated in the no talking scenario were Staphylococcal species (38/75, 51%). In the speech scenarios, 8 oral flora organisms were isolated with of 4 of the cases in the no face mask scenario, and 4 cases in the tight-fitting face mask without adhesive tape scenario. Causative oral flora organisms included 3 colonies of *Streptococcus mitis*, 3 colonies of *Streptococcus viridans*, and 2 colonies of undifferentiated Streptococcus.

DISCUSSION

THE COVID-19 PANDEMIC HAS NECESSITATED UNIVERSAL face mask protocols for infection control, which has resulted in significant interest in understanding face mask effectiveness from various disciplines,[11,15–17] including ophthalmology.[18] However, there are few data on how patient face mask use may alter bacterial dispersion during an intravitreal injection, which subsequently may affect the risk of postinjection endophthalmitis. In this experimental study of bacterial dispersion during a simulated intravitreal injection scenario with 6 different face masks, we found that subjects wearing tight-fitting face masks without adhesive tape covering the superior portion of the mask grew the most bacterial organisms under both no talking and speech scenarios. However, the introduction of adhesive tape to secure the superior portion of the same tight-fitting face mask significantly reduced the amount of bacterial dispersion toward the eyes. Furthermore, the introduction of adhesive tape to secure the superior portion of a tight-fitting face mask resulted in no statistically significant difference in bacterial dispersion compared with bacterial dispersion with an N95 face mask.

We assessed the number of CFUs under no talking conditions to simulate the clinical practice of a no talking policy in which intravitreal injections are administered under a strict policy of silence, such that the physician, patient, and others in the room do not speak during the injection procedure. Under the no talking condition, bacterial growth around the subject's eye was highest when wearing a tight-fitting face mask without adhesive tape, and the source of bacteria in these no talking scenarios may be from the subject's natural breathing. A known physiologic reaction to stress and anxiety is to hyperventilate, which some patients routinely do as they are anticipating an intravitreal injection—wearing a mask may contribute to this phenomenon. However, bacterial growth was significantly reduced when subjects wore the same tight-fitting face mask but had adhesive tape attached over the entire superior portion of the face mask. The bacterial growth was reduced such that the addition of adhesive tape to the tight-fitting face mask resulted in similar rates of bacterial growth compared with those wearing an N95 face mask.

A previous study on face mask use by the injector during simulated intravitreal injections reported that subjects speaking without a face mask resulted in an increased proportion of bacterial colony growth.[8] The current study evaluated bacterial growth under speech scenarios where subjects were instructed to read a 2-minute standardized script while wearing various face masks. Regardless of the type of face mask worn, subjects in the speech scenarios had significantly greater bacterial growth compared with the no talking scenarios. Specifically, subjects wearing no face masks had greater bacterial growth in the speech scenario compared with the no talking scenario. These findings underscore the importance of a speech reduction policy when intravitreal injections are administered. Furthermore, within the various speech scenarios, subjects wearing N95 face masks and tight-fitting face masks with adhesive tape had significantly fewer CFUs compared with subjects wearing no face masks, loose face masks, and tight-fitting face masks without tape. Current guidelines from the U.S. Centers for Disease Control and Prevention at the time of writing recommend cloth face covering, which may not adhere to the face as well.[14] Our study found similar rates of bacterial dispersion with cloth-based face mask use in both scenarios. Although we did not assess the effect of adhesive tape for cloth face masks, future studies may be indicated to evaluate these specific scenarios given the findings of this study.

In both the no talking and speech scenarios, subjects wearing a tight-fitting face mask without tape had the highest bacterial growth—similar to, or even higher than, not wearing any mask. It is possible that the tight-fitting face without tape may result in a greater amount of bacterial dispersion upward toward the subject's eye.[12,17] Indeed, a recent study assessing respiratory droplet velocities with face mask use during simulated coughs reported that even with tight-fitting face masks, small openings can lead to leakage of droplets around the mask.[17] However, our study suggests that securing the superior portion of the same, tight-fitting face mask with adhesive tape significantly decreased the amount of bacterial growth, suggesting that securing the superior portion of the mask with tape may create an important barrier for upward bacterial dispersion.

Although all forms of endophthalmitis are visually threatening, oral flora–associated endophthalmitis is associated with a particularly poor prognosis.[19–21] Previous clinical studies have established that oral flora–associated endophthalmitis may be reduced with the implementation of a strict no talking policy by the physician and patient during intravitreal injection administration.[7,21] Refraining from speaking during an intravitreal injection is thought to minimize the potential to contaminate the uncapped needle or conjunctival surface with oral flora immediately before or during the injection. Indeed, in our study, there were no cases of oral flora isolated during the no talking scenarios, which further supports the efficacy of a speech reduction policy to reduce the risk of oral flora–associated endophthalmitis. In contrast, during the speech scenarios, oral flora were isolated when subjects were speaking without a face mask or speaking with a tight-fitting face mask without tape, which parallel a previous study showing high rates of oral flora growth during similar scenarios.[8]

Our study has several limitations. Blood agar plates were used for bacterial quantification and identification. However, these plates do not precisely reproduce the target field of the ocular surface. Furthermore, we standardized the distance between the agar plate and the eye, the duration of speech, and the positioning of the face mask, but in real-world clinical scenarios there may be significant variability in all of these clinical factors. For example, previous studies have reported that the tendency to wiggle one's face beneath a surgical mask[12] may increase bacterial dispersion and shedding, presumably from the beard and facial skin. Another limitation is that the speech scenarios had the subject read a script for 2 minutes, which is likely more time than any patient would spend speaking during an intravitreal injection. However, 2 minutes may be realistic when considering the preparation time for an intravitreal injection during which the patient may be speaking. Furthermore, previous studies have suggested that bacterial dispersal can occur even with shorter durations of speech.[22] Another limitation is that our study included participants who were previously fitted to wear N95 face masks, which may not be generalizable to the true patient population who wears N95 masks. Our findings may be underestimating the real-world benefit of taping because many patients come in with suboptimal fitting masks, regardless of material or type. Finally, it is unknown if the statistically significant difference in colony counts among the groups is of clinical significance. This study cannot prove that the presence of additional bacterial colonies surrounding the injection site necessarily contributes to an increased risk of postinjection endophthalmitis. However, given the devastating visual prognosis of postinjection endophthalmitis, it is critical to minimize the potential risk of endophthalmitis with any measures available.

In summary, these experiments replicate the specific conditions of an intravitreal injection when patients are wearing different types of face masks. Until now, there was minimal evidence in the literature to guide practitioners in the management of patient face mask use during an intravitreal injection. These in vitro experiments suggest that addition of adhesive tape to the superior portion of a patient's face mask during an intravitreal injection reduces bacterial dispersion, which may subsequently reduce the risk of postinjection endophthalmitis.

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