# RESEARCH



# Microbiological comparison of the disinfecting efficacy of small and large cotton swabs in nasotracheal intubation: a randomized trial

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# Abstract

**Background** Nasotracheal intubation (NTI) is necessary during surgeries requiring clear access to the surgical field and in patients with respiratory issues. This study investigates the pre-NTI nasal disinfection efficacy of different cotton swab sizes, hypothesizing that smaller swabs could minimize bleeding while maintaining disinfection efficacy.

**Methods** Patients classified as American Society of Anesthesiologists-physical status (ASA-PS) class 1 or 2 scheduled for general anesthesia with NTI were randomly assigned to either a large cotton swabs (LCS) or fine cotton swabs (FCS) group in this randomized controlled trial (RCT)." After anesthesia, a fine cotton swab was inserted into the inferior nasal meatus in both groups to collect bacteria (sample A). Next, the nasal cavity was disinfected with LCS or FCS according to the patient group. Bacteria were collected by inserting a fine cotton swab into the inferior nasal meatus (sample B). After surgery, bacteria were collected from the endotracheal tube tip using a fine cotton swab in both groups (sample C). The samples were cultured for 24 hours, and the colonies from samples A–C were counted. The changes in bacteria count between samples A and B and samples A and C were determined. Nasal bleeding from cotton swab insertion was assessed as a secondary outcome. Student's t-tests, a chi-square independence test, and Mann–Whitney U tests were used for the statistical analysis. The statistical significance level was set at p < 0.05.

**Results** Between samples A and B, the change in bacteria count was 7.2% (1.4–26.1%) (median[interquartile range]) in the LCS group and 6.9% (0.9–22%) in the FCS group (p = 0.90). Between samples A and C, the change in bacteria count was 7.5% (0.2–44%) in the LCS group and 8.3% (0.3–39%) in the FCS group (p = 0.55). We examined 62 subjects in each group (LCS and FCS), and samples A, B, and C were collected from all participants in both groups. Nasal bleeding occurred in 42/62 in the LCS group and 22/62 in the FCS group (p < 0.01).

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**Conclusion** Cotton swab thickness did not impact disinfection efficacy, but large swabs increased the risk of nasal bleeding. We recommend FCS for nasal disinfection prior to NTI in ASA 1–2 patients, as they reduce bleeding risk without compromising disinfection.

Trial registration UMIN-CTR (registration no. UMIN000051495), June 30, 2023.

Keywords Benzalkonium chloride, Cotton swab, Nasal bleeding, Nasotracheal intubation

# Background

Nasotracheal intubation (NTI) is commonly required in dental, maxillofacial, and oropharyngeal surgeries as it provides the surgeon with better access to the surgical field [1]. NTI is also used in patients with respiratory insufficiency, intensive care unit patients requiring prolonged airway maintenance, and patients for whom orotracheal intubation is challenging due to trismus. However, NTI can lead to complications such as nasal bleeding [2, 3], bacteremia [4], retropharyngeal perforation [5], and partial or complete tube obstruction [6, 7]. Reports that have linked NTI to bacteremia [8, 9] suggest it is likely caused by the translocation of intranasal bacteria into the respiratory tract, which can potentially lead to sepsis [4, 10]. Therefore, preventing nasal bleeding and disinfecting the nasal mucosa before NTI is crucial to minimize anesthesia-related mortality.

Previous research has compared the disinfection efficacy of povidone-iodine and benzalkonium chloride (BZK) and found that BZK is most suitable for NTI disinfection [11]. However, the use of large cotton swabs for disinfection was found to pose a risk of increased nasal bleeding. A recent study has proposed a new disinfection method in which Magill forceps are used to temporarily remove the endotracheal tube from the patient's oral cavity. Any dirt is wiped away with a clean cotton swab, and then the tube is reintroduced into the oral cavity for nasotracheal intubation [12]. However, this method is not commonly used and there is debate over the best approach. Therefore, in this study, we focused on the size of the cotton swab used for disinfection. We hypothesized that smaller cotton swabs could provide adequate disinfection while reducing the risk of nasal bleeding. Using BZK in both groups, we compared the disinfection efficacy of large (14 mm diameter) cotton swabs (LCS) with that of fine (6 mm diameter) cotton swabs (FCS).

# Methods

# Ethics approval and consent to participate

This study was approved by the Ethics Committee of the School of Dentistry, Aichi Gakuin University (approval no. 678), and was prospectively registered as a clinical trial with UMIN-CTR on June 30, 2023 (registration no. UMIN000051495, https://center6.umin.ac.jp/cgi-open-bin/ctr/ctr\_view.cgi?recptno=R000058701). The study adheres to CONSORT guidelines and was conducted in

accordance with the tenets of the 2013 revision of the Declaration of Helsinki. The first patient was recruited on July 6, 2023. All patients provided written informed consent to participation.

# Participants

Patients aged 20–70 years, classified as American Society of Anesthesiologists-physical status (ASA-PS) class 1 or 2, and scheduled for general anesthesia with NTI, were enrolled in this study. Patients who did not consent and those who had an allergy to BZK were excluded. Patients were randomly assigned to two groups based on the disinfection method."

To minimize bias and enhance the reliability of results, the randomization was performed by a researcher not involved in this study, using a computer-generated random number table. This was a single-blind study, with only the participants blinded to their group. The evaluators (the anesthesiologists and the microbiologists) could not be blinded because they were able to discern the thickness of the swabs used.

# Anesthesia, sample collection, and microbial count

The same method of anesthesia induction was used for all patients, with no premedication. Patients independently walked to one of three operating theaters where the procedures were performed, where standard vital monitors (electrocardiogram, blood pressure, and oxygen saturation) were applied. Anesthesia was induced using propofol (1–2 mg/kg), remifentanil (0.2  $\mu$ g/kg/min), and fentanyl (100  $\mu g$ ), with rocuronium (0.6 mg/kg) as the neuromuscular blocking agent. Mask ventilation with 100% oxygen was performed until the neuromuscular blocking agent took effect. During mask ventilation, the patient's inferior nasal passage was swabbed with a sterile fine cotton swab (Hakuzo Medical, Osaka, Japan).before disinfection (sample A). Subsequently, the anterior nare was disinfected with a large cotton swab and tramazoline nasal drops were administered to the nasal cavity. After disinfecting the lower nasal passages through the nostrils using a large or fine cotton swab (Hakuzo Medical, Osaka, Japan) depending on the patient group (LCS or FCS), a fine cotton swab was inserted into the lower nasal passage (in both groups) to collect bacteria (sample B). BZK (Zalkonin<sup>®</sup> solution 0.025, Kenei Pharmaceutical Co., Ltd, Osaka, Japan) was used at normal clinical

concentrations of 0.025% and NTI was performed after the muscle relaxant was observed to take effect. To minimize variability related to technical skill, all intubations were performed by experienced anesthesiologists.In all cases, we maintained general anesthesia using total intravenous anesthetic. After the surgery was completed and the endotracheal tube had been extubated, the internal surface of the endotracheal tube (1 cm from the tip) was swabbed immediately with a fine cotton swab in both groups to collect sample C.

Our focus was on establishing how much of the bacteria from the upper airway that had invaded the lower airway during intubation had been suppressed by disinfection. If we had swabbed the outer surface of the endotracheal tube, we would have been unable to avoid contamination by nasal bacteria during extubation. Therefore, we swabbed just the internal surface of the endotracheal tube.

After the specimens were collected, the swab heads were removed. A and C samples were immersed in 10 ml of sterile physiological saline, while B samples were immersed in 40 ml of sterile physiological saline to dilute the absorbed disinfectant (BZK). The samples were then refrigerated and submitted for analysis within 6 h. Viable microorganisms in the swab samples were quantified using a culture technique. The samples were vigorously vortexed at maximum speed for 30 s to release the microbes from the swab heads into the saline. Following the removal of the swab heads, the samples were centrifuged at 4,000  $\times$  g for 15 min at 4 °C to concentrate the microbes. The resulting pellets were resuspended in 1 ml of saline. Serial dilutions of the suspensions were then prepared, and 50 µl of each dilution was spread onto agar plates. Brain heart infusion agar (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA) was utilized to determine the total microbial count.

# **Evaluation of parameters**

The primary outcome was disinfection efficacy, determined by changes in the bacteria counts. The number of bacteria per cotton swab (in Colony forming Unit: CFU) in samples A, B, and C were evaluated, and the rates of change in bacterial count (B/A, C/A) were calculated. CFU stands for Colony Forming Unit, which represents the number of bacteria capable of forming colonies when cultured on an agar plate.

The secondary outcome was the presence of nasal bleeding. Nasal bleeding was defined as the presence of blood on the cotton swab after disinfecting the inferior nasal passage with either the large or fine cotton swab.

The clinical and demographic variables assessed were patient sex, height, weight, age, anesthesia duration, and surgical duration.

# Statistical analysis

We intended to investigate 129 patients based on a power analysis. We calculated the minimum number of samples required for our results to have sufficient power (total n = 116 cases, 58 per group; effect size, 0.52;  $\alpha$ -error, 0.05; power, 0.80). The effect size was derived from the statistical results of a pilot study that used the patient distribution for the post-disinfection change in bacteria count as a standard (LCS group, 10 cases; FCS group, 10 cases). We calculated our final sample size requirement, with a simple but adequate adjustment for dropouts provided by  $Nd = N/(1-R)^2$ , where a dropout rate of R is expected, N is the sample size calculated assuming no dropout, and Nd is the required sample size with dropouts [13]. The dropout rate in a preliminary study was 0.05. Therefore, our adjusted sample size requirement was 128.5, and 128 patients were finally assessed.

Student's t-tests were used to assess the effects of age, height, weight, anesthesia duration, and surgical duration. A chi-square independence test  $m \times n$  contingency table was used to assess the effects of sex and the presence or absence of nasal bleeding. Mann–Whitney U tests were used to assess the number of bacteria found in samples A, B, and C and the rates of change in bacterial count between samples A and B and samples A and C. Per protocol analysis was used. The level of statistical significance was set at p < 0.05.

# Results

From July 2023 to April 2024, A total of 129 patients, aged 20–70 years, classified as American Society of Anesthesiologists-physical status (ASA-PS) class 1 or 2, and scheduled for general anesthesia with NTI, were enrolled in this study. Patients who did not consent (n=1) and those who had an allergy to BZK were excluded. The patients were randomly assigned to two groups based on the disinfection method. These were the LCS group (n=64, with two patients discontinued) and the FCS group (n=64, with two patients discontinued). In total, 124 patients were assessed. Figure 1 shows a CONSORT flow diagram of patient recruitment. 128 participants were randomly assigned to one of two groups based on disinfection with either LCS or FCS. Four participants dropped out during the trial.

Table 1 shows patient sex, height, weight, age, anesthesia time, and operation time. Both the LCS and FCS groups showed similar anesthetic and surgical durations, and no significant differences were found in terms of other perioperative outcomes.

Table 2 shows the bacterial counts from samples A, B, and C and the rates of change in these counts for B/A and C/A.



Fig. 1 CONSORT flowchart of the patient recruitment process. One patient was excluded before randomization because we did not obtain their consent to participate in the study

Table 1	Patients'	background

	LCS (n=62)	FCS (n=62)	<i>p</i> value	95% CI
Male/Female	30/32	33/29	0.58 NS	
Height (cm)	$163.3 \pm 9.7$	164.2±8.4	0.50 NS	-4.1-2.3
Weight (kg)	$60.2 \pm 12.0$	$59.7 \pm 13.4$	0.80 NS	-3.9-5.1
Age (year)	$35.7 \pm 12.7$	$33.7 \pm 11.4$	0.37 NS	-2.3-6.1
Anesthesia time (min)	$115.9 \pm 53.9$	$121.5 \pm 56.0$	0.56 NS	-24.9-13.7
Operation time (min)	$70.3 \pm 42.7$	77.4±53.4	0.41 NS	-24.1-9.8
Values are mean+stand	ard deviation o	r number. NS: N	ot significa	ant: LCS: Large

cotton swabs; FCS: Fine cotton swabs; CI: Confidence interval

Table 2 The effect of LCS or FCS for nasal bacteria

	LCS	FCS	p value
A (CFU)	6200 (1300–40400)	12,500 (2640–58250)	0.10 NS
B (CFU)	340 (60–1600)	1030 (205–4055)	0.02
C (CFU)	32 (30–2730)	1520 (65–5390)	0.07 NS
B/A (%)	7.2 (1.4–26.1)	6.9 (0.9–22)	0.90 NS
C/A (%)	7.5 (0.2–44)	8.3 (0.3–39)	0.55 NS

Values are median (Quartile 25th and 75th percentiles). NS: Not significant; LCS: Large cotton swabs; FCS: Fine cotton swabs; CFU: Colony-forming unit

# Sample A (before disinfection)

The median bacterial count was 6,200 CFU/swab in the LCS group and 12,500 CFU/swab in the FCS group. Despite the numerical difference, this was not statistically significant (p=0.10), suggesting that the baseline bacterial load was similar between the two groups.

# Sample B (after disinfection)

The bacterial count reduced by 7.2% (1.4–26.1%) in the LCS group and 6.9% (0.9–22%) in the FCS group (p=0.90), indicating that both large and fine cotton swabs achieved similar levels of bacterial reduction after disinfection with no significant difference between the two groups.

# Sample C (post-surgery, from the endotracheal tube)

The bacterial count reduction between sample A and C was 7.5% (0.2–44%) in the LCS group and 8.3% (0.3–39%) in the FCS group, with no significant difference (p=0.55). This suggests that both types of swabs had a comparable effect on reducing bacterial translocation during intubation.

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Table 3 shows the frequency of nasal bleeding in the two groups. Nasal bleeding was observed in 42/ 62 patients in the LCS group and 22/ 62 patients in the FCS group. This was a statistically significant between-group difference (p=0.0003).

# Discussion

In this RCT study, we compared the disinfectant efficacy of nasal cavity disinfection using LCS and FCS. The results revealed no significant differences in the disinfectant efficacy between the two swab sizes, but there was a clear difference in the incidence of nasal bleeding.

# Comparison of disinfectant efficacy

First, concerning the disinfectant efficacy of the LCS group and FCS group, no significant differences were observed between the two groups in the reduction of bacteria (B/A and C/A) after disinfection with BZK. This suggests that the thickness of the cotton swab has a limited impact on disinfectant efficacy, with the effectiveness of the disinfectant itself being the predominant factor.

# Incidence of nasal bleeding

Although no clinical complications were observed, the incidence of nasal bleeding was significantly higher in the LCS group (p<0.01)."Nasal bleeding resulting from NTI is primarily due to damage to the venous plexus in the nasal mucosa and the curvature of the nasal septum. Adenoid hypertrophy is more commonly associated with pharyngeal bleeding [14]."Nasal bleeding during NTI can compromise airway patency and lead to serious complications like airway obstruction. Ongoing bleeding can also impact the surgical field. Therefore, all possible measures must be taken to prevent bleeding. Several methods of preventing epistaxis have been reported [14, 15]. These include tramazoline, which was used in both groups in this study.

Bacteremia refers to the presence of bacteria in the bloodstream. Specifically, nasal bleeding can facilitate the entry of bacteria from the nasal cavity into the bloodstream, which could theoretically increase the risk of bacteremia. There are several reports on the association between tracheal intubation and bacteremia. Konstantinou et al. [16] performed blood culture tests by taking 10 ml of blood 10 min after orotracheal intubation, and found that 12.0% of the subjects had bacteremia, with a higher proportion of difficult intubation cases, which they identified as a risk factor for bacteremia. Similarly,

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	LCS (n=62)	FCS (n=62)	<i>p</i> value
Presence	42	22	0.0003
Absence	20	40	

Values are number. LCS: Large cotton swabs; FCS: Fine cotton swabs

Valdés et al. [17] conducted bacterial culture tests on blood samples taken 30 s after nasotracheal and orotracheal intubation in 110 subjects, reporting that 11.7% of nasotracheal intubations and 12.0% of orotracheal intubations showed positive bacterial cultures. However, we acknowledge that the direct link between NTI and sepsis requires further investigation.

Cotton swabs with a larger diameter result in greater friction against the nasal mucosa, making bleeding more likely. In contrast, FCS, with their smaller diameter and increased flexibility, exert less pressure on the nasal mucosa, resulting in a lower risk of bleeding. Although NTI is a widely performed method of securing the airway, the procedure is invasive, and discomfort to the patient must be minimized as much as possible. As our results showed no difference in disinfectant efficacy between the two cotton swab sizes, it is preferable to choose smaller, less invasive swabs. Disinfection with thinner cotton swabs is particularly beneficial for older patients and those with fragile nasal mucosa.

# Selection of disinfectants

BZK was used as the disinfectant in this study. It was not necessary to examine how different disinfectants, such as povidone-iodine or chlorhexidine, affect disinfectant efficacy and the mucosa as such research already exists and the utility and efficacy of BZK in NTI has already been reported [11]. In vitro, BZK has been shown to inhibit the growth of *Staphylococcus aureus* even at 2<sup>9</sup> dilutions. Moreover, BZK maintains its threshold concentration even after inactivation or diffusion and dilution over time [11]. The present study also showed a high disinfection effect from BZK, regardless of the thickness of the cotton.

# Clinical applications, limitations, and future research directions

The results of this study support the recommendation of fine cotton swab use for NTI in clinical settings. They may also contribute to the standardization of NTI procedures that maximize the effective removal of bacteria from the nasal cavity while reducing patient burden. However, our study had some limitations. First, regarding bacterial counts,

# Major variability was observed between patients

Various factors can contribute to such variations, particularly the dryness of the mucosal membrane in the nasal cavity. Moving forward, it may be essential to adjust for mucosal membrane dryness before collecting specimens.

Additionally, while the differences in bacterial counts between the LCS and FCS groups in samples A, B, and C were not statistically significant, it is important to consider potential reasons for these variations. Possible explanations include differences in surgery types, the use of different operating rooms, or even the variability among anesthesiologists or examiners. Notably, all nasal swab sampling was performed by a single examiner, which should have minimized variation due to sampling technique. However, further research is needed to explore whether other factors, such as the duration of surgery or anesthesia, may have influenced these differences.

Second, since only the nasal cavities and the inferior nasal passages were disinfected, we cannot exclude the possibility that contamination from the oropharyngeal region during intubation influenced our results. In particular, the results could have been significantly affected by the duration of surgery or anesthesia. In the future, large-scale clinical trials without these limitations are expected to establish the superiority of thin cotton swabs and to improve the safety of NTI.

We compared the thickness of the swabs only, but their shape and material may also affect disinfectant efficacy and patient comfort. Research into considerations such as whether the tip is rounded or pointed, and the density or hardness of the cotton, could lead to further improvements.

Finally, we did not assess the specific microbial species present in the bacterial cultures, focusing instead on overall bacterial load. Future studies should examine microbial species to provide a more detailed understanding of the disinfection process and potential implications for NTI safety.

# Conclusion

Cotton swab thickness did not impact disinfection efficacy, but large swabs increased the risk of nasal bleeding. FCS are recommended for nasal disinfection prior NTI as they reduce bleeding risk without compromising disinfection.

# Abbreviations

- NTI Nasotracheal intubation
- FCS Fine cotton swabs
- LCS Large cotton swabs
- CFU Colony Forming Unit

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# Author contributions

AS, MH, and NT helped with substantial contributions to the conception or design of the work, as well as statistics, planning, coding, reporting, and writing the manuscript. YK (Yuji Kamimra), YS, and EK contributed substantially to the conception or design of the work and the interpretation of the study results. MO and KS (Kyoko Shida) helped with substantial contributions to the conception or design of the work, as well as the conduct and reporting of the work. HH, ST, and HK helped with substantial contributions to the conception or design of the work, statistics, and manuscript writing. TS and YK (Yuka Kikuchi) contributed substantially to the conception or design of the work, as well as to statistics and manuscript writing. KS (Kazuya Sobue) and YH contributed substantially to the conception or design of the work, supervision of the manuscript, and development of the overall study.

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The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

# Data availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

# Declarations

# Ethics approval and consent to participate

This study was approved by the Ethics Committee of the School of Dentistry, Aichi Gakuin University (approval no. 678), and was prospectively registered as a clinical trial with UMIN-CTR on June 30, 2023 (registration no. UMIN000051495, https://center6.umin.ac.jp/cgi-open-bin/ctr/ctr\_view .cgi?recptno=R000058701). The study adheres to CONSORT guidelines and was conducted in accordance with the tenets of the 2013 revision of the Declaration of Helsinki. The first patient was recruited on July 6, 2023. All patients provided written informed consent to participation.

# **Consent for publication**

Not applicable.

# **Competing interests**

The authors declare no competing interests.

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