A phase 2 trial of a topical antiseptic bundle in head and neck cancer surgery: Effects on surgical site infection and the oral microbiome

Joseph Zenga,a* Samantha Atkinson,b,c Tina Yen,a Becky Massey,a Michael Stadler,a Jennifer Bruening,a William Peppard,a,c,d Michael Reuben,f Michael Hayward,b,g,h Brian Mesich,† Blake Buchan,† Nathan Ledeboer,† Joyce L. Sanchez,‡ Raphael Fraser,† Chien-Wei Lin,† Mary L. Holtz,b,g,h Musaddiq Awan,l Stuart J. Puram,m Sidharth V. Puram,n and Nita Salzmanb,g,h

aDepartment of Otolaryngology, Division of Head and Neck Surgical Oncology and Reconstruction, Medical College of Wisconsin, Milwaukee, WI, United States
bCenter for Microbiome Research, Medical College of Wisconsin, Milwaukee, WI, United States
cDepartment of Microbiology & Immunology, Medical College of Wisconsin, Milwaukee, WI, United States
dDepartment of Surgery, Division of Surgical Oncology, Medical College of Wisconsin, Milwaukee, WI, United States
eDepartment of Pharmacy, Medical College of Wisconsin, Milwaukee, WI, United States
fDepartment of Pharmacy, Children’s Wisconsin, Milwaukee, WI, United States
gDepartment of Pediatrics, Division of Gastroenterology, Medical College of Wisconsin, Milwaukee, WI, United States
hChildren’s Research Institute, Children’s Wisconsin, Milwaukee, WI, United States
iDepartment of Pathology, Medical College of Wisconsin, Milwaukee, WI, United States
jDepartment of Medicine, Division of Infectious Diseases, Medical College of Wisconsin, Milwaukee, WI, United States
kDepartment of Biostatistics, Medical College of Wisconsin, Milwaukee, WI, United States
lDepartment of Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI, United States
mDepartment of Medicine, Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, United States
nDepartment of Otolaryngology, Division of Head and Neck Surgical Oncology and Reconstruction, Washington University School of Medicine, Saint Louis, MO, United States

Summary

Background Head and neck cancer (HNC) surgery remains an important component of management but is associated with a high rate of surgical site infection (SSI). We aimed to assess the safety and efficacy of a topical mucosal antiseptic bundle in preventing SSI and evaluate microbial predictors of infection through a genomic sequencing approach.

Methods This study was an open-label, single-arm, single-center, phase 2 trial of a topical mucosal antiseptic bundle in patients with HNC undergoing aerodigestive tract resection and reconstruction. Patients underwent topical preparation of the oral mucosa with povidone-iodine (PI) and chlorhexidine gluconate (CHG) pre- and intra-operatively followed by oral tetracycline ointment every 6 hours for 2 days post-operatively. The primary outcome was change in bacterial bioburden at the oral surgical site. Secondary outcomes included safety, SSI, and microbial predictors of infection.

Findings Of 27 patients screened between January 8, 2021, and May 14, 2021, 26 were enrolled and 25 completed the study. There were no antiseptic-related adverse events. The topical mucosal antiseptic bundle significantly decreased oral bacterial colony-forming units from pre-operative levels (log_10 mean difference 4.03, 95%CI 3.13–4.92). There were three SSI (12%) within 30 days. In correlative genomic studies, a distinct set of amplicon sequence variants in the post-operative microbiome was associated with SSI. Further, despite no instance of post-operative orocervical fistula, metagenomic sequence mapping revealed the oral cavity as the origin of the infectious organism in two of the three SSI.

Interpretation The bacterial strains which subsequently caused SSI were frequently identified in the pre-operative oral cavity. Accordingly, a topical antiseptic bundle decreased oral bacterial bioburden throughout the peri-operative period and was associated with a low rate of SSI, supporting further study of topical antisepsis in HNC surgery.

*Corresponding author at: Division Chief, Don and Sharyn Blatnik Professor of Head and Neck Surgical Oncology and Reconstruction, Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, 9200 W. Wisconsin Ave. Milwaukee, WI 53226, United States.
E-mail address: jyzenga@mcw.edu (J. Zenga).
Introduction

Head and neck cancers (HNC), including tumors of the oral cavity, pharynx, and larynx, remain an important source of cancer-related mortality worldwide, with an estimated 66,000 cases and over 14,000 associated deaths in the United States in 2021 alone.1 HNC surgery requiring vascularized reconstruction continues to be a central component of management for select primary and recurrent tumors. However, a main source of peri-operative morbidity after HNC surgery is surgical site infection (SSI), which has been consistently reported in over 20% of cases.2-4 Post-operative SSI leads to substantial physical, psychosocial, and financial burdens on patients, providers, and hospital systems.5 Importantly, SSI may also lead to delays in necessary post-operative radiation, which can result in an increased risk of cancer recurrence and decreased overall survival.6-8

While several clinical risk factors for SSI have been identified, many are largely immutable including extent of cancer resection, prior radiotherapy, age, and comorbidity.9 For these reasons, interventional efforts to decrease SSI for HNC have primarily investigated the choice or duration of intravenous antibiotic therapy.3 Intravenous prophylactic antibiotics are well known to decrease the incidence of SSI in HNC, with infection rates over 70% in early placebo-controlled trials.10-14 Despite the substantial benefit of short-term therapy, however, delivering intravenous antibiotics for more than 24 hours post-operatively has not been shown to improve SSI rates, which have seen little change over the past four decades of study.1

In HNC surgery, there are multiple opportunities for bacterial contamination of the surgical field leading to SSI. For cancer resection, incisions are required in both the pharyngeal mucosa and cervical skin with intraoperative connections between the aerodigestive tract and the neck soft tissues. These wounds must then be...
repaired with vascularized skin flap reconstructions taken from elsewhere on the body. While common upper aerodigestive tract microbiota can cause SSI infections, infections are often due to pathogens not typically found among commensal oronasal or skin bacteria, including Methicillin-resistant Staphylococcus aureus, Pseudomonas, Enterobacter, and Enterococcus spp.\textsuperscript{4,15-17} It remains unclear whether these bacteria are present pre-operatively in upper aerodigestive tract environment or are introduced peri-operatively from another source.

However, the clear benefit of intravenous antibiotics in the immediate peri-operative period but not beyond 24 hours suggests that the target organisms leading to infection are present at the surgical site in the early peri-operative period. Therefore, considering the high baseline microbial bioburden of the oral cavity and its proximity to the operative field in HNC surgery, we hypothesized that oral contamination is the primary mechanism leading to SSI and that the oral microbiome may play a role in susceptibility to infection. To test this hypothesis, this trial evaluates the impact of peri-operative topical mucosal decontamination on the oral microbioburden and oral microbiome composition at the surgical site throughout the peri-operative period and to evaluate its effects on SSI. Further, to more precisely determine the source of infection, whole genome sequencing was performed of the organisms isolated from SSI purulence with subsequent mapping of these sequences back on to the metagenomic microbial communities of the pre-operative and post-operative oral cavity.

Methods

Study design and participants

This study was an open-label, single-arm, single-center interventional Phase 2 trial assessing the safety and antibacterial efficacy of a topical mucosal antiseptic bundle in HNC patients undergoing upper aerodigestive tract resection. Patients were eligible for enrollment if they were 18 years or older undergoing an open surgical procedure requiring a communication between the upper aerodigestive tract and cervical skin repaired with a vascularized flap reconstruction, which could be either a regional pedicled and/or free flap. Patients were excluded if they had an allergy to study medications or had an active infection at the time of surgery.

Ethics

The study was done in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines and was approved by the relevant Medical College of Wisconsin Scientific Review and Institutional Review Board committees (NCT04721626). All patients provided written informed consent before study entry.

Topical mucosal antiseptic bundle and clinical procedures

Enrolled patients received a topical mucosal antiseptic bundle consisting of pre-operative, intra-operative, and post-operative agents (Table 1). The topical antiseptic bundle included three agents – povidone-iodine (PI), chlorhexidine gluconate (CHG), and tetracycline – based on survey results of current surgical practice across the United States, evidence from related surgical subspecialties, and existing data in HNC surgery.\textsuperscript{18-23} For all cases, the pre-operative topical antiseptics were applied to the entire upper aerodigestive tract including the oral cavity and pharynx. Further, intra-operative irrigations covered all exposed oral and pharyngeal mucosa. Intravenous antibiotic therapy was given to all patients prior to surgery with standard intra-operative redosing and for 24 hours post-operatively, in accordance with national and institutional guidelines.\textsuperscript{24} Ampicillin/sulbactam was the preferred intravenous agent as per institutional standard of care. Pre-operative skin preparation was performed in accordance with CDC guidelines.\textsuperscript{24}

Microbial specimen collection

Microbial sample collection procedure

Fresh samples were taken for quantitative and qualitative microbiology as well as bacterial genomic assessments. The following sites and time-points were selected to represent a comprehensive investigation of the peri-operative microbial environment:

- 1. Pre-operatively (under anesthesia prior to topical therapies)
- 2. Oral cavity
- 3. Nasal cavity
- 4. Intra-operatively (after tumor resection)
- 5. Oral cavity before antiseptic wound irrigation
- 6. Oral cavity after antiseptic irrigation
- 7. Surgical drapes adjacent to the operative field
- 8. Post-operatively (day 3-5 after surgery)
- 9. Oral cavity
- 10. From patient’s hospital room environment in the follow areas: door handle, overbed table, suction tubing.

Swabs were de-identified prior to transfer to laboratory staff who were blinded to patient identification. To maintain consistency between patients and collection time-points, all specimens were collected by the same study author (JZ), based on established methodology.\textsuperscript{25} For sampling methodology and swab processing for genomic and microbiologic analyses, see Supplement.
Outcomes

The primary endpoint was the quantitative change in abundance of bacterial bioburden in the oral cavity in response to the topical antiseptic therapies. Secondary endpoints included SSI, adverse events (AEs, NCI CTCAE v5.0) due to the topical antiseptic intervention, features in the oral microbiome associated with SSI (for definition, see Supplement), and genomic mapping to identify the site of origin of the infectious organism.

Statistical analysis

Quantitative microbiologic culture. With anticipated enrollment of 25 patients, the detectable effect size (group mean difference) with 80% power using a paired t-test with a significance level of 5% is 0.6 times the standard deviation of the group difference of bacterial abundance. A paired-samples t-test was conducted to compare bacterial loads at pre-operative, intra-operative, and post-operative time-points. In this analysis, the intra-operative and post-operative time-points were compared separately to the pre-operative values. All paired-samples analyses were done on the log₁₀ scale and used a 5% level of statistical significance. Prior to conducting the analysis, the assumption of normally distributed difference of log bacterial load was confirmed using the skew and kurtosis levels as well as quantile-quantile plots.

Genomic and metagenomic analyses. 16S rDNA analysis was used to evaluate the composition of the oral microbiome at multiple peri-operative time-points and to determine if features of the oral microbiome were associated with SSI. Whole genome sequencing (WGS) was performed on bacteria isolated from culture of purulence expressed from the SSI. In the patients who developed SSI, metagenomic sequencing was then performed on the bacterial communities isolated from peri-operative swabs. Additionally, any pathogenic bacteria that were cultured from the peri-operative microbiologic samples with a species-level match to the SSI organism also underwent WGS. To identify the peri-operative origin of the organism which led to SSI, its genomic sequence was mapped back on to the WGS or metagenomic data from the peri-operative environment. In the case where multiple WGS samples were obtained from the same bacterial species in the same patient, a pangenome analysis was performed to determine if differences existed between these strains. For bioinformatics analyses, see Supplement.

Role of the funding source. The study sponsor had no role in study design, in collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

Results

Clinical outcomes

Twenty-seven patients were screened between January 8, 2021, and May 14, 2021. Twenty-six were enrolled and 25 completed the study (Figure 1). Patients were followed for 30 days post-operatively for the development of SSI. There were no antiseptic-related AEs. All patients received prophylactic intravenous ampicillin/sulbactam for 24 hours. All patients underwent post-operative tube feeding and remained NPO for at least 7 days. Baseline characteristics are shown in Supplementary Table 1. There were three cases of SSI (12%), all of which occurred as a purulent infection which spontaneously drained through the cervical incision. SSI was identified at a median of 18 days post-operatively (range 15-25 days). There were no orocervical or pharyngocervical fistulae and all patients who developed SSI had passed a videofluoroscopic swallow study. Baseline and treatment characteristics of the study population are shown in Supplementary Table 1.

Microbiologic and genomic correlates

Swabs from all seven peri-operative sites and time-points for all 25 included study patients (see above,
Microbial sample collection procedure along with swabs of purulent fluid sampled from each of the three SSI underwent qualitative and quantitative microbiologic culture and DNA extraction.

Microbiologic culture analysis

Incidence of pathogenic bacteria found in the pre-operative oral cavity. The pre-operative oral cavity was found to harbor pathogenic organisms in five patients (20%) including *Serratia marcescens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella variicola*, and *Klebsiella oxytoca*. Of these patients, the pathogen persisted in the post-operative oral culture in two cases (*S. marcescens* and *P. aeruginosa*). Of these patients, only one (*P. aeruginosa*) went on to experience an SSI which grew that same pathogen.

Overall effects of the topical antiseptic bundle on decontamination of the oral cavity surgical site. The topical antiseptic bundle significantly decreased oral bacterial colony-forming units (CFU) overall from pre-operative baseline to post-operative sampling (log₁₀ mean difference 4.03, 95%CI 3.13–4.92, Table 2). This difference in oral bacterial bioburden in response to topical antiseptic therapy (74.1 times the standard deviation of the group difference) exceeded the targeted change in the primary endpoint (0.6 times the standard deviation of the group difference). Pre-operative and intra-operative topical mucosal antisepsis both exhibited a significant decrease in CFU counts, while the post-operative topical antiseptic regimen alone did not demonstrate a significant independent effect.

SSI and the microbiology of infection. The three cases of SSI among study patients grew *Staphylococcus epidermis*, *Eikenella sp.*, and *P. aeruginosa*, respectively. When culture results from all sites and time-points were evaluated, the same bacterial species which caused SSI was identified in a peri-operative culture for only one patient (*P. aeruginosa*, found in the pre-operative and post-operative oral cavity, Table 3). In the other two cases of SSI, the bacterial species causing infection was not found in any peri-operative microbiologic culture.

Genomic and metagenomic correlates. While DNA extraction was performed for all 178 unique samples collected in this study, due to low recovery of bacterial genomic material from several sites (intra-operative oral cavity, surgical drapes, post-operative hospital environment), only the nasal cavity and the pre-operative and post-operative oral swabs subsequently underwent DNA sequencing.

16S sequencing: effects of topical antisepsis on the oral microbiome and association with SSI. 16S rDNA sequencing was performed in all study patients on pre-
operative and post-operative oral swabs to evaluate the impact of topical mucosal antisepsis on the oral microbiome and its association with SSI. Measures of alpha- and beta-diversity demonstrated a significant increase in the microbial diversity in the post-operative period, suggesting oral re-colonization with diverse bacteria after surgery (Figure 2). To determine specific changes in microbial taxa, linear discriminant analysis effect size (LEfSe) was performed.26 Several orders of pathogenic bacteria were identified which were significantly enriched in the oral cavity in the pre-operative or intra-operative periods, including Pseudomonales and Enterobacterales, which were subsequently less prevalent in the post-operative samples (Supplementary Figure 2). Specific features of the pre-operative and post-operative oral microbiomes were then assessed for association with SSI. While features of the pre-operative microbiome were not found to significantly predict SSI, a unique set of 21 amplicon sequence variants from the post-operative microbiome were significantly associated with SSI (model accuracy 0.8, AUC 0.75, Supplementary Figure 3). In particular, the combined presence of two organisms, Actinomyces sp and Pseudomonas formosensis, in the post-operative microbiome represented the most important identified predictors of SSI.

Genomic and metagenomic sequencing: source-tracking of the infectious organisms. In cases of SSI, we attempted genomic mapping of the infectious organism to identify where in the peri-operative environment that strain may have originated. To achieve this, the bacterial strain which was isolated from the purulence of the SSI was sub-cultured from glycerol stock and underwent WGS. WGS was successful in two of the three cases of SSI. In one case (S. epidermidis) the organism could not be re-cultured from glycerol stock and, therefore, could not undergo WGS and further analysis. Next, genome sequencing was performed on previously extracted and preserved bacterial DNA from the nasal and oral swabs for each patient. Of the two cases with available WGS for the infectious organism, one (Eikenella sp) underwent metagenomic sequencing of the peri-operative nasal and oral samples. In the second case, the same species (P. aeruginosa) was cultured from the SSI and both the pre-operative and post-operative oral swabs and, therefore, these samples were sub-cultured from glycerol stock and underwent WGS for comparison to the infectious organism via pangenome analysis.

Sequencing mapping and pangenome analyses revealed the oral cavity as the origin of the infectious organism in the two analyzable cases. In the case of SSI with Eikenella sp, despite not being identified by microbiologic culture in the peri-operative environment, the WGS mapped specifically to the pre-operative oral microbiome and was not found in the nasal cavity or post-operative oral microbiome (Figure 2). In the case of SSI with P. aeruginosa, pangenome analysis of the WGS revealed all samples had identical genome sequences apart from three singletons lost from the infectious organism and post-operative isolate. While PSI-BLAST search revealed that the function of these genes is unknown, there was an associated increased resistance to tetracycline identified in P. aeruginosa from the post-operative oral sample and SSI, as compared with the pre-operative isolate.

Discussion

Upfront surgery remains an essential component in the management of HNC. For many patients with advanced oral cavity or laryngeal/hypopharyngeal cancers, open resection with vascularized flap reconstruction continues to maximize oncologic control. Further, with the increasing use of non-surgical therapies for organ

| Intervention assessed                      | Oral microbial samples compared                     | N  | Log10 mean difference | 95% confidence interval | P-value |
|-------------------------------------------|----------------------------------------------------|----|-----------------------|-------------------------|---------|
| Pre-operative decolonization (PI/CHG)     | Pre-operative prior to decolonization vs Intra-operative prior to antiseptic irrigation | 25 | 1.5755                | 1.1563 - 1.9948         | <.0001  |
| Intra-op antiseptic irrigation (PI/CHG)   | Intra-operative before antiseptic irrigation vs Intra-operative after antiseptic irrigation | 25 | 2.3798                | 1.6823 - 3.0773         | <.0001  |
| Post-op topical antisepsis (tetracycline) | Intra-operative after antiseptic irrigation vs Post-operative after topical antiseptic | 25 | 0.0729                | -0.8848 - 1.0305        | 0.8765  |
| Overall topical antiseptic bundle         | Pre-operative prior to decolonization vs Post-operative after topical antiseptic | 25 | 4.0282                | 3.1344 - 4.9219         | <.0001  |

Table 2: Change in oral bacterial bioburden in response to topical mucosal antisepsis.
preservation for other head and neck disease stages and subsites, greater numbers of patients with locoregional cancer recurrence or intractable complications related to radiotherapy will similarly require open upper aerodigestive tract resection and reconstruction for oncologic or functional indications. All such cases come with a significant risk of post-operative SSI, which results in immense physical and financial strain on patients, providers, and hospital systems.5,27

Despite more than four decades of extensive study on risk factors for SSI and the optimization of prophylactic intravenous antibiotic regimens, there has been little change in infection rates after HNC surgery.3 Although numerous clinical risk factors have been identified, the microbial features associated with infection remain poorly understood. However, a microbial approach to understanding SSI after HNC surgery may lead to advances beyond what has been accomplished through study of clinical risk factors. In particular, this prospective clinical trial illuminated three key avenues through which a microbial approach may lead to improvements in preventing SSI after HNC surgery: (1) decreasing oral bacterial bioburden, (2) understanding the impact of the oral microbiome on SSI, and (3) source-tracking of the infectious organisms which lead to SSI.

**Decreasing oral bacterial bioburden.** The results of this trial demonstrated that oral mucosal topical antisepsis significantly decreased bacterial bioburden at the oral surgical site and is most effective in the pre-operative and intra-operative settings. While certain opportunistic pathogenic species did persist through treatment (P. aeruginosa, S. marcescens), topical antiseptic therapy significantly decreased overall oral CFU counts and eradicated 60% of opportunistic pathogenic bacteria identified in the pre-operative oral cavity. The overall quantitative decrease in oral bacterial abundance in response to topical antiseptic therapy exceeded our pre-study targeted change. These findings are significant because in HNC surgery the oral cavity becomes continuous with the surgical wound and neck soft tissues after tumor resection. While the threshold level required to affect SSI rates remains uncertain, a significant decrease in oral bacterial bioburden and pathogenic isolates may lead to decreased wound contamination and fewer SSI. Further, as demonstrated by genomic source-tracking in this study, for many cases of SSI the causative organism is present in the oral cavity pre-operatively and leads to infection despite no clinically apparent post-operative orocervical fistula. This finding suggests that pathogen eradication through topical mucosal therapies may translate to fewer clinical SSI. A final important consideration in the use of topical antiseptics to decrease wound bioburden is the impact of non-oral aerodigestive tract surgical sites (pharynx and larynx). In these cases, although the oral cavity may not be directly violated, the adjacent pharyngeal mucosa

| Pre-operative oral prior to topical mucosal antisepsis | Intra-operative nasal prior to intra-operative antiseptic irrigation | Intra-operative oral prior to intra-operative antiseptic irrigation | Intra-operative surgical drapes | Post-operative oral | Post-operative hospital room environment |
|-----------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------------------------------|-------------------|-----------------------------------------|
| Normal oral flora | No growth | Growth of oral flora | No growth | Normal oral flora | No growth |
| Normal oral flora | Normal nasal flora* | Normal nasal flora* | Normal oral flora | Normal oral flora | Normal oral flora |
| Staphylococcus epidermidis | 1. Normal nasal flora* | 2. Staphylococcus epidermidis | 1. Normal oral flora | Pseudomonas aeruginosa | Ampicillin/sulbactam resistant |
| Eikenella sp | No growth | Growth of oral flora | No growth | Proprionibacterium acnes | Tetracycline sensitive |
| 2. Staphylococcus | Ampicillin/sulbactam resistant | Ampicillin/sulbactam | | | |
| 1. Normal nasal flora | | | | | |
| 2. Proprionibacterium | | | | | |
| acnes | | | | | |

| Normal oral flora | Normal oral flora* | Normal nasal flora | Normal oral flora |
|-------------------|-------------------|-------------------|-------------------|
| 1. Normal oral flora | 2. Pseudomonas aeruginosa | Ampicillin/sulbactam resistant |
| | | Tetracycline sensitive |

*Eikenella sp not identified.

### Table 3: SSI microbiology and whether the same organisms were identified in peri-operative microbiologic culture.

> www.thelancet.com Vol 81 Month July, 2022 7
and associated oral salivary flow becomes continuous with the neck soft tissues. For this reason, our protocol included pre-operative and intra-operative coverage of the entire exposed upper aerodigestive with the topical antiseptic agents for all cases. We hypothesize that, given the continuity of the oral cavity and pharynx, the microbial risk factors for SSI will be similar for oral and non-oral surgical subsites.

Source-tracking of infectious organisms. Through a novel application of a genomic approach to oncologic surgery, this clinical trial has shed light on the peri-operative origin of organisms that lead to SSI after HNC surgery. In the two analyzable cases of SSI in this trial, both were found to have originated from the pre-operative oral cavity. Strikingly, SSI occurred with these bacterial strains despite the absence of detectable levels of these organisms intra-operatively after oral decontamination and without the development of a clinically apparent orocervical fistula. This finding most likely suggests that minimal levels of these bacterial strains persisted through topical oral decontamination, subsequently seeding the neck soft tissues either intra-operatively or in the early post-operative period.

The impact of the oral microbiome. Despite a major reduction in overall bacterial counts from pre-operative levels, there appeared to be no clear adverse effect on the microbiome reconstitution. The post-operative oral microbiota demonstrated greater diversity than matched pre-operative samples with fewer pathogenic bacterial orders, including Pseudomonales and Enterobacterales. However, despite this greater diversity and fewer overall pathogenic organisms, several features of the post-operative microbiome were found to correlate with SSI. Specifically, the combined presence of P. formosensis and Actinomyces sp were identified as a significant risk factor for SSI in two of the three cases. While little is known about P. formosensis, the presence of Actinomyces colonization has been associated with delayed oral wound healing in previous reports. These findings suggest the possibility that certain genera may create a post-operative oral milieu conducive to infection.

In summary, this study provides preliminary evidence that the pre-operative oral cavity commonly harbors the bacterial strains which will ultimately lead to infection, that a topical oral antiseptic bundle may be beneficial in limiting infection rates, and that the composition of the post-operative oral microbiome may play a role in susceptibility to SSI after HNC surgery. The primary limitations of this study relate to available sample size, low frequency of SSI resulting in only two analyzable cases for genomics, and a single-arm design. Another important limitation was the inability to recover sufficient bacterial genomic material from the skin, surgical drapes, and hospital environment. These sites have very low bacterial counts and may require additional methodological optimization in future studies to achieve more reliable metagenomic yield. The absence of these genomic data do limit the strength of our conclusions about the origin of the bacterial organisms that lead to infection. However, given that the pre-operative oral cavity was identified as the most likely source, any presence of the infectious organism at an additional peri-operative site would most likely represent contamination from the oral cavity. Additionally, this trial involved a specific antiseptic bundle which may not be generalizable to other antiseptic regimens.

Figure 2. Genomic mapping of the whole genomic sequence of the Eikenella sp which caused surgical site infection on to the metagenome of the pre-operative nasal cavity, pre-operative oral cavity, and post-operative oral cavity. The genome of the infectious organism closely aligns with an Eikenella sp organism identified in the pre-operative oral environment but is not found in the metagenome at the other sites. X-axis represents each gene in the Eikenella sp genome. Y-axis represents mean coverage for each gene.
Further, given the persistence of select pathogens through treatment and the development of antibiotic-resistance in one case, these results provide the basis to refine the topical regimen to both better eradicate pre-existing pathogens while also limiting the development of drug resistance. Finally, this study also established the feasibility of applying genomic methodology to oncologic surgery for tracking bacterial strains which causeSSI in the peri-operative period and could be extrapolated to study the etiology of infection in other surgical disease sites and specialties.

Contributors
All authors contributed significantly to conception, design, analysis and interpretation of data. All authors performed critical editing and final approval of the manuscript. BB and BMe had full access to the microbiologic data, wrote microbiologic study plan, and performed the microbiologic analyses and interpretation. SA had full access to the genomic data, performed all the bioinformatics analyses, and wrote the bioinformatics methods and results. RF and CWL had full access to clinical study data, wrote the biostatistical plan, and performed all the biostatistical analysis for the microbiologic and clinical investigations. All other manuscript sections were drafted by JZ. All study patients were treated clinically by JZ and BMa, both of whom have accessed and verified the data, and are responsible for the decision to submit the manuscript.

Data sharing statement
Individual level de-identifiable trial data and protocol will be shared on request with publication for researchers with a methodologically sound proposal after obtaining Proposals should be directed to jyzen ga@mcw.edu.

Declaration of interests
None.

Acknowledgements
This work was supported by the Alliance National Cancer Institute Community Oncology Research Program (NCORP) Research Base grant 7UG1CA189823 (PI: Zenga) as well as the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number UL1TR001436. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. The authors thank the University of Wisconsin Biotechnology Center DNA Sequencing Facility for providing 16S, whole genome, and metagenomic sequencing services.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104099.

References
1. American Cancer Society. Cancer statistics: analysis tool. https://cancerstatisticscenter.cancer.org. Accessed 9 April 2019.
2. Mitchell RM, Mendez E, Schmitt NC, Bhranay AD, Futran ND. Antibiotic prophylaxis in patients undergoing head and neck free flap reconstruction. JAMA Otolaryngol Head Neck Surg. 2015;141(1):109–115. https://doi.org/10.1001/jamaoto.2015.0531.
3. Vila PM, Zenga J, Jackson RS. Antibiotic prophylaxis in clean-contaminated head and neck surgery: a systematic review and meta-analysis. Otolaryngol Head Neck Surg. 2017;157(4):386–395. https://doi.org/10.1177/0194599817712125.
4. Veve MP, Greene JB, Williams AM, et al. Multicenter assessment of antibiotic prophylaxis spectrum on surgical infections in head and neck cancer microvascular reconstruction. Otolaryngol Head Neck Surg. 2018;159(4):59–67. https://doi.org/10.1177/0194599818849999.
5. Penel N, Lefebvre JL, Cazin Jl, et al. Additional direct medical costs associated with nosocomial infections after head and neck cancer surgery: a hospital-perspective analysis. Int J Oral Maxillofac Surg. 2008;37(2):113–119. https://doi.org/10.1016/j.ijoms.2007.08.002.
6. Graboyes EM, Garrett-Mayer E, Ellis MA, et al. Effect of time to initiation of postoperative radiation therapy on survival in surgically managed head and neck cancer. Cancer. 2017;123(24):4841–4850. https://doi.org/10.1002/cncr.30539.
7. Goel AN, Frangos MJ, Raghavan G, et al. The impact of treatment package time on survival in surgically managed head and neck cancer in the United States. Oral Oncol. 2019;01(88):39–48. https://doi.org/10.1016/j.oraloncology.2018.11.021.
8. Chen MM, Harris JF, Oroco RK, Sirjani D, Harz W, Divi V. Association of time between surgery and adjuvant therapy with survival in oral cavity cancer. Otolaryngol Head Neck Surg. 2018;158(6):1051–1056. https://doi.org/10.1177/0194599818773879.
9. Cannon RB, Houlton JJ, Mendez E, Futran ND. Methods to reduce postoperative surgical site infections after head and neck oncology surgery. Lancet Oncol. 2017;18(7):e405–e413. https://doi.org/10.1016/S1470-2045(17)30575-3.
10. Raine CH, Bartzokas CA, Stell PM, Gallaway A, Corkill JE. Chem prophylaxis in major head and neck surgery. J R Soc Med. 1984;77(12):1006–1009.
11. Anjani S, Martin J, Lal A, et al. Antibiotic prophylaxis for preventing surgical-site infection in plastic surgery: an evidence-based consensus conference statement from the American association of plastic surgeons. Plast Reconstr Surg. 2015;135(6):1723–1739. https://doi.org/10.1097/PRS.0000000000001652.
12. Sagarin R, Odel PF, Pultiqin IF. Antibiotic prophylaxis in head and neck cancer surgery. J Otolaryngol. 1988;17(4):78–80.
13. Mandell-Brown M, Johnson JT, Wagner RL. Cost-effectiveness of prophylactic antibiotics in head and neck surgery. Otolaryngol Head Neck Surg. 1984;92(5):526–531.
14. Johnson JT, Yu VL, Myers EN, Muder RR, Theare PB, Diven WF. Efficacy of two third-generation cephalosporins in prophylaxis for head and neck surgery. Arch Otolaryngol. 1984;110(4):224–227.
15. Durand ML, Yarlagadda BB, Rich DL, et al. The time course and microbiology of surgical site infections after head and neck free flap surgery. Laryngoscope. 2015;125(10):1858–1869. https://doi.org/10.1002/lary.25038.
16. Yang CH, Chew KY, Solomkin JS, Lin PY, Chiang YC, Kuo YR. Surgical site infections among high-risk patients in clean-contaminated head and neck reconstructive surgery: concordance with preoperative oral flora. Ann Plast Surg. 2015;75(1):55–56. https://doi.org/10.1097/SAP.0000000000000406.

www.thelancet.com Vol 81 Month July, 2022 9
17 Lin S, Mekk S, Lisgaris MV, Ahadizadeh EN, Zender CA. Post-operative MRSA infections in head and neck surgery. *Am J Otolaryngol*. 2017;38 (4):417–421. https://doi.org/10.1016/j.amjoto.2017.01.015.

18 Fournel I, Tiv M, Soulis M, Hua C, Astruc K, Aho Gléle IS. Meta-analysis of intraoperative povidone-iodine application to prevent surgical-site infection. *Br J Surg*. 2010;97(11):1603–1613. https://doi.org/10.1002/bjs.7312.

19 Goutok M, Terzi MC, Egel T, Arslan NC, Canda AE. Does wound irrigation with chlorhexidine gluconate reduce the surgical site infection rate in closure of temporary loop ileostomy? A prospective clinical study. *Surg Infect (Larchmt)*. 2018;19(6):614–619. https://doi.org/10.1089/sur.2018.061.

20 Funahara M, Yamaoto S, Ueda M, et al. Prevention of surgical site infection after oral cancer surgery by topical tetracycline: results of a multicenter randomized control trial. *Medicine (Baltimore)*. 2017;96 (48):878. https://doi.org/10.1097/MD.0000000000000889.

21 Stabenau KA, Akakpo KE, Richmond JD, et al. Postoperative wound infections in head and neck surgery: the current state of antiseptic and antibiotic practices. *Oral Oncol*. 2021;118:105361. https://doi.org/10.1016/j.oraloncology.2021.105361.

22 Schweizer ML, Chuang HT, Septimus E, et al. Association of a bundled intervention with surgical site infections among patients undergoing cardiac, hip, or knee surgery. *JAMA*. 2015;313 (21):2162–2171. https://doi.org/10.1001/jama.2015.5387.

23 Grandis JR, Vickers RM, Rihs JD, Yu VL, Johnson JT. Efficacy of topical amoxicillin plus clavulanate/ticarcillin plus clavulanate and clindamycin in contaminated head and neck surgery: effect of antibiotic spectra and duration of therapy. *J Infect Dis*. 1994;170 (1):729–732. https://doi.org/10.1093/infdis/170.3.729.

24 Berrios-Torres SI, Umscheid CA, Braitler DW, et al. Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017. *JAMA Surg*. 2017;152(8):784–791. https://doi.org/10.1001/jamasurg.2017.0904.

25 Consortium HMP. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–214. https://doi.org/10.1038/nature11234.

26 Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011;12(6):R60. https://doi.org/10.1186/gb-2011-12-6-r60.

27 Sweeney L, Rosenthal EL, Light T, et al. Outcomes and cost implications of microvascular reconstructions of the head and neck. *Head Neck*. 2019;41(4):930–939. https://doi.org/10.1002/hed.25424.

28 Silva PGB, de Codes E, Freitas MO, Martins JOL, Alves APNN, Sousa FB. Experimental model of oral ulcer in mice: comparing wound healing in three immunologically distinct animal lines. *J Oral Maxillofac Pathol*. 2018;22(3):444. https://doi.org/10.4103/jomfp.JOMFP_444_17.