

Navigating the microbial community in the trachea-opharynx of breast cancer patients with or without neoadjuvant chemotherapy (NAC) *via* endotracheal tube: has NAC caused any change?

Hee Yeon Kim¹, Tae Hyun Kim¹, Jeong Hwan Shin², Kwangrae Cho³, Heon-Kyun Ha⁴, Anbok Lee⁴ and Young Jin Kim⁵

¹ Department of Surgery, Busan Paik Hospital, Inje University, Busan, South Korea

² Department of Laboratory Medicine and Paik Institute for Clinical Research, Inje University, Busan, South Korea

³ Department of Anesthesiology and Pain Medicine, Busan Paik Hospital, Inje University, Busan, South Korea

⁴ Department of Surgery, Chung-Ang University Gwangmyeong Hospital, Chung-Ang University College of Medicine, Chung-Ang University, Gyeonggi-do, South Korea

⁵ Department of Laboratory Medicine, Kyung Hee University College of Medicine, Kyung Hee University Medical Center, Seoul, South Korea

ABSTRACT

Background: We compare the diversity and niche specificity of the microbiome in the trachea-opharynx microbiome of malignant breast neoplasm with or without neoadjuvant chemotherapy (NAC) *via* NGS analysis.

Methods: We prospectively collected a total of 40 endotracheal tubes intubated from subjects, of whom 20 with NAC treated breast cancer (NAC group) and 20 with breast cancer without NAC (Non-NAC group). We generated 16S rRNA-based microbial profiles in Illumina™ platform and alpha diversity indices were compared between groups. For the comparison of taxa abundance, linear discriminant analysis effect size method with Kruskal-Wallis test was used. The distribution of variables between the two groups was compared using the Mann-Whitney test. For beta diversity analysis, PERMANOVA was used.

Results: Among the diversity indices, the NAC group showed significantly lower Chao1, Inverse Simpson, and Shannon indices than the Non-NAC group. The three most frequent taxa of all two groups were *Streptococcus* (20.4%), followed by *Veillonella* (11.9%), and *Prevotella* (10.4%). This order was the same in NAC and non-NAC groups.

Conclusion: Here, we provide the first comparison data of the respiratory tract microbiome of breast cancer patients with or without NAC *via* NGS analysis. This study ultimately seeks to contribute to future studies on the lower respiratory tract in cancer patients with cytotoxic chemotherapy by establishing reliable control data.

Submitted 20 April 2023
Accepted 6 October 2023
Published 23 November 2023

Corresponding authors
Anbok Lee, ab-lee@hanmail.net
Young Jin Kim, khmclab@gmail.com

Academic editor
Jun Chen

Additional Information and
Declarations can be found on
page 8

DOI 10.7717/peerj.16366

© Copyright
2023 Kim et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Microbiology, Molecular Biology, Oncology, Respiratory Medicine, Medical Genetics
Keywords Breast cancer, Lower respiratory tract, Microbiome, Neoadjuvant chemotherapy

INTRODUCTION

The human microbiome refers to the collection of all bacterial flora in the human body (Mammen & Sethi, 2016). Microbial communities represent the unique characteristics of specific body habitats, with their configuration and roles varying across and within organs (Costello et al., 2009). The respiratory tract is exposed to 7,000 L of air per day and 10^4 – 10^6 /m³ microorganisms (Kumpitsch et al., 2019). Therefore, the microbiome niche in the respiratory tract is composed of diverse resident and transient bacterial communities (Dickson et al., 2016; Kumpitsch et al., 2019).

Advances in metagenomic analyses have made it possible to obtain complete or nearly complete genomic sequences from uncultured bacteria. Next-generation sequencing (NGS) is a large-scale method that can be used to comprehensively analyze the human microbiome community under various conditions (Garza & Dutilh, 2015). Recent studies performed using NGS-based microbiome analysis suggested that an imbalance in the normal flora, a state known as dysbiosis, is associated with a broad spectrum of diseases, from sepsis to cancer development (Chen et al., 2019).

Among living women diagnosed with cancer in the past 5 years, 7.8 million had breast cancer, making it the most prevalent cancer in 2020 (World Health Organization (WHO), 2020). Neoadjuvant chemotherapy (NAC) is currently the treatment of choice for locally advanced breast cancer (Rubovszky & Horvath, 2017). Recent NGS-based microbiome analyses revealed that the microbiota may be altered in patients with breast cancer receiving NAC. Moreover, increasing evidence suggests that chemotherapy can cause microbiota dysbiosis, which may harm the safety and efficacy of NAC (Iida et al., 2013; Liu et al., 2021; Nadeem et al., 2021). The gut microbiome has been linked to NAC in patients with breast cancer, which may affect the treatment response and post-NAC weight changes (Chapadgaonkar, Bajpai & Godbole, 2023; Wu et al., 2022). The oral microbiome has also been reported to undergo changes following anticancer chemotherapy in studies involving pediatric patients with various types of cancer (Proc et al., 2022). Therefore, regarding the significance of microbial composition in NAC, few studies have focused on the impact of breast cancer NAC on the respiratory microbiome (Chen et al., 2019). In this study, we compared the lower respiratory tract microbiome of patients with breast cancer who were treated or not treated with NAC.

MATERIALS AND METHODS

Patients

Patients aged 19–64 years who underwent surgery or other procedures under general anesthesia using an endotracheal tube (ETT) for breast disease between March 2020 and October 2021 were included in the study. Patients with breast cancer were classified into two groups: the NAC group consisting of patients with breast cancer who were treated with NAC, and the non-NAC group consisting of patients with breast cancer who did not receive prior chemotherapy. Patients with the following conditions were excluded: those with a history of antimicrobial exposure within 3 months, those with respiratory lesions,

those with current respiratory disease, smokers, patients with diabetes, patients with anemia (hemoglobin less than 8 g/dL), and patients with an intubation time of at least 8 h.

Specimen and clinical information collection

Before anesthesia, the patients were asked to gargle the oral cavity with 20 mL of distilled water for 30 s and place the solution in a 50 mL Falcon tube. After surgery and anesthesia, the ETT was removed from the bronchus and aseptically cut at 10 cm from the distal end. The distal part exposed to the lower airway was placed in a 50 mL Falcon tube. Collected samples were immediately transported to the laboratory and washed with DNA-free water using a vortex mixer. A negative control sample was prepared by placing an unused endotracheal tube in a Falcon tube and washing with DNA-free water. The washed solution was stored at -80°C until analysis. The time from extubation to storage did not exceed 60 min.

The following medical records were reviewed: sex, age, height, body weight, hemoglobin level, current diagnosis, chemotherapy history, surgical procedure, surgery time, smoking history, diabetes, tuberculosis, and pneumonia.

Amplicon sequencing analysis and bioinformatics

Nucleic acids were extracted using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Libraries were prepared according to the 16S Metagenomic Sequencing Library protocol (Illumina, San Diego, CA, USA). The V3-V4 region of the 16S rRNA gene was amplified using the V3-F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and V4-R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' primers, which contained an overhang adapter. PCR products were purified using AMPure beads (Agencourt Bioscience, Beverly, MA, USA). Purified PCR products were used for sequencing library construction using Herculase II Fusion DNA Polymerase and Nextera XT Index Kit V2 (Illumina). The PCR products were qualified using an Agilent Technologies 2100 (Agilent Technologies, Santa Clara, CA, USA) with a TapeStation D1000 ScreenTape (Agilent Technologies). The libraries were sequenced on a MiSeq (Illumina) using a 600-cycle kit (2×300 bp), and paired-end FASTQ files were generated. Sequencing adapter and F/R primer sequences were removed using Cutadapt (v3.2) (Martin, 2011). Error correction steps, including quality filtering, denoising, merging, and chimeric sequence removal, were performed using R software (v4.0.3; The R Project for Statistical Computing, Vienna, Austria) with the DADA2 (v1.18.0) package, and amplicon sequence variants were prepared (Callahan et al., 2016). For comparative analysis of the microbial communities, normalization was performed by subsampling based on the number of reads of the sample with the minimum read number among all samples using QIIME (v1.9) (Caporaso et al., 2010). Each amplicon sequence variant was taxonomically assigned using BLAST+ (v.2.9.0) to the reference database (NCBI 16S rDNA database May 18, 2021) (Camacho et al., 2009). If the query coverage was less than 85% or identity of the matched area was less than 85%, the taxonomy was not assigned. The criteria and procedures for taxonomic assignment based on BLAST+ are

described in the [Supplemental file](#). QIIME (v.1.9) was used to calculate bacterial community diversity indices such as Shannon, inverse Simpson, and Chao1.

Statistical analysis

The linear discriminant analysis effect size method was used to compare taxa abundances between the two groups. The alpha value for the factorial Kruskal-Wallis test among classes was 0.05, and the threshold for the log-scale linear discriminant analysis score was two ([Segata et al., 2011](#)). The Mann-Whitney test was used to compare the distribution of alpha diversity indices between the two groups and to compare clinical data between groups using MedCalc version 11.5.1.0 (MedCalc Software, Ostend, Belgium). A p value of less than 0.05 was considered to indicate statistically significant results. For beta diversity analysis, Bray-Curtis dissimilarity was determined using PERMANOVA in the Vegan package in R ([Adloff et al., 2003](#); [Dixon, 2003](#)).

Ethics statement

This study was approved by the Medical Ethics Committee of Inje University Busan Paik Hospital (IRB # BPIRB 2019-01-112-003), and written consent was obtained from all participants.

RESULTS

Clinicopathological characteristics of patients and clinical specimens

Forty relevant specimens were collected from female patients in the NAC group ($n = 20$) and non-NAC group ($n = 20$). The median (interquartile range) age of the 40 subjects was 50 (44–55) years, and the age distribution did not significantly differ between the two groups ($P = 0.096$). The pre-chemotherapy clinical stage of the NAC group was significantly more advanced than that of the non-NAC group ($P < 0.001$). Neither group showed significant differences in clinicopathological characteristics, except for the pre-chemotherapy clinical stage.

In the NAC group, the NAC regimen for breast cancer included the following: a combination of pertuzumab, trastuzumab, docetaxel, and cyclophosphamide; a combination of doxorubicin and cyclophosphamide followed by docetaxel; and a concurrent combination of doxorubicin and docetaxel. The median (interquartile range) duration of chemotherapy and number of days from completion of cytotoxic chemotherapy to sample collection were 108 (105–120) and 22 (20–27) days, respectively.

The median operative time for the 40 patients was 177 min. The patient characteristics are summarized in [Table 1](#).

Genus level taxon by study group

After subsampling, the number of reads per sample was 46,125. A total of 169 genus-level taxa was found in all samples. Of these, 32 taxa showed a relative abundance of 2% or more in at least one sample ([Fig. 1](#)). The numbers of genus-level taxa were 145 and 123 in the NAC and non-NAC groups, respectively. The most frequent taxon (median, %) across all samples was *Streptococcus* (20.4%), followed by *Veillonella* (11.9%) and *Prevotella* (10.4%).

Table 1 Clinical characteristics of the patients by study groups.

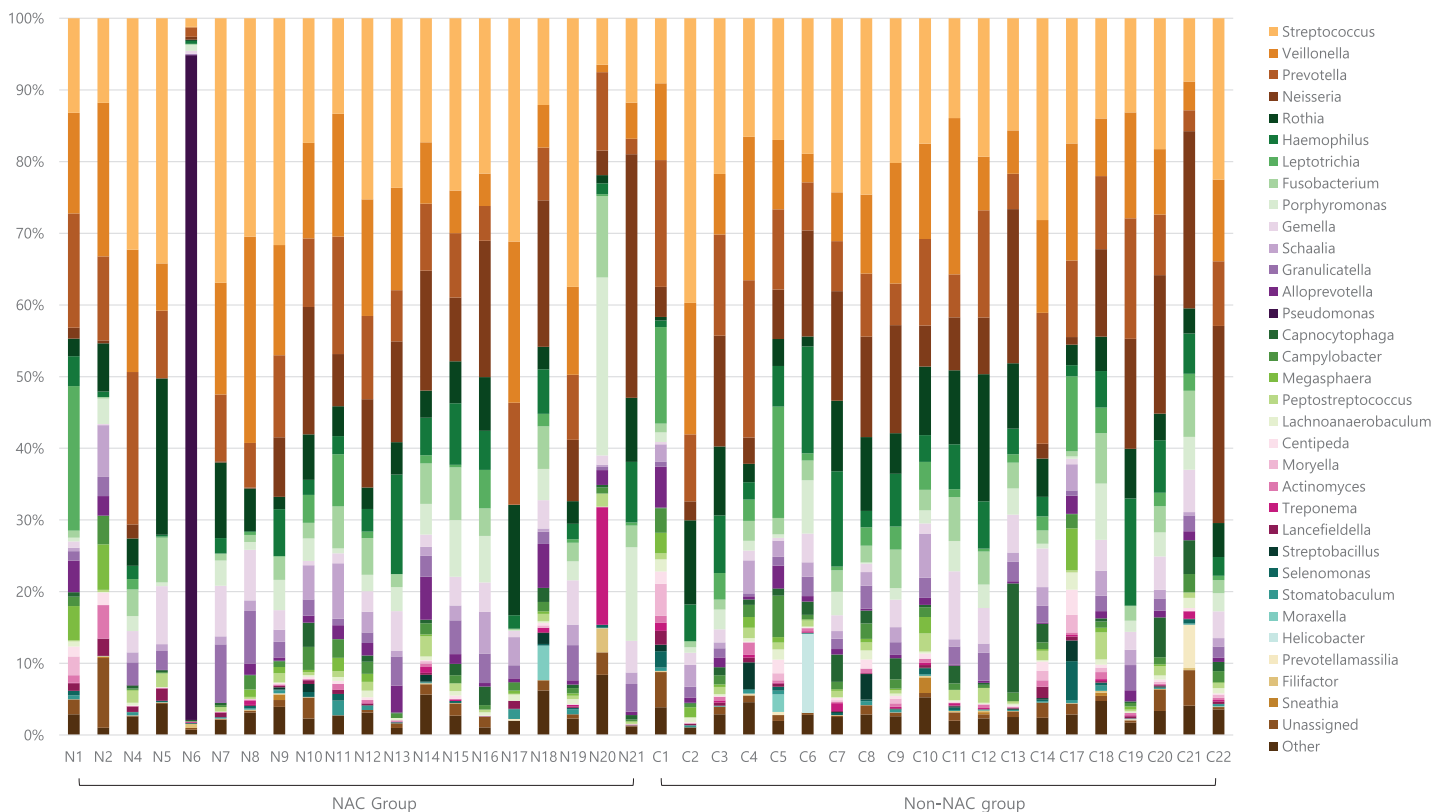
	NAC (n = 20)	Non-NAC (n = 20)	Total (n = 40)	P
Age (years)	53 (48–59)	46 (43–53)	50 (44–55)	0.096
Body mass index	24.3 (21.8–26.9)	22.8 (21.3–24.8)	23.7 (21.4–25.5)	0.209
Serum glucose (mg/dL)	105 (100–113)	105 (100–114)	105 (100–113)	0.756
Intubated time (minutes)	175 (160–225)	182 (153–213)	177 (160–217)	0.850
Duration of chemotherapy (days)	108 (105–120)	N/A		
Days from last chemotherapy	22 (20–27)	N/A		
Pre-chemotherapy clinical stage				<0.001***
cI	0	12		
cII	9	8		
cIII	11	0		

Notes:

Abbreviations: NAC, Neoadjuvant chemotherapy; N/A, not applicable.

***P < 0.001 are considered as statistically significant.

Continuous variables are reported as median (interquartile range).

**Figure 1** Genus level taxa found in endotracheal tube washed fluid of patients with breast cancer. Taxa with a relative abundance of at least 2% in at least one sample were marked as individual taxa and others were pooled as others. [Full-size DOI: 10.7717/peerj.16366/fig-1](https://doi.org/10.7717/peerj.16366/fig-1)

This order was the same in the NAC (22.7%, 13.7%, and 9.4%, respectively) and non-NAC (17.9%, 10.9%, and 9.8%, respectively) groups. In linear discriminant analysis effect size analysis, *Tannerella*, *Actinomyces*, *Centipea*, *Moryella*, *Streptobacillus*, *Leptotrichia*, and

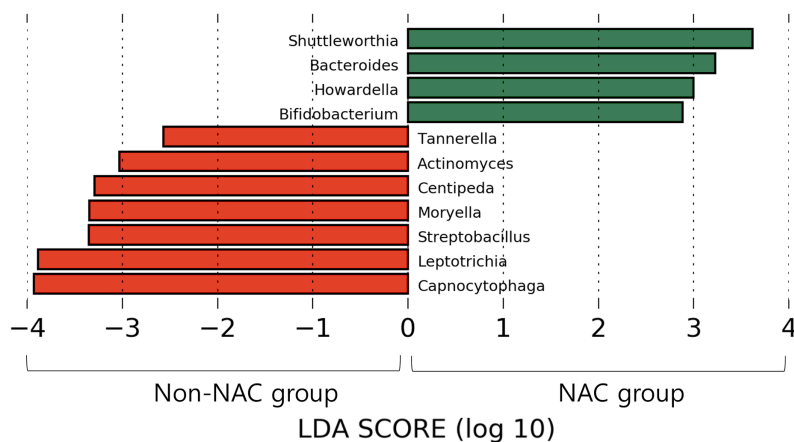


Figure 2 Microbiota comparison between Non-NAC group (red) and NAC (green) group. Taxa shows significant differences in linear discriminant analysis score comparisons.

Full-size DOI: 10.7717/peerj.16366/fig-2

Table 2 Comparison of diversity indices between study groups.

Diversity indices	NAC	Non-NAC	<i>p</i>
Chao1	188.7 (164.2–232.3)	227.8 (212.2–255.9)	0.047*
Inverse Simpson	0.940 (0.927–0.956)	0.959 (0.945–0.966)	0.008**
Shannon	5.227 (4.745–5.549)	5.670 (5.272–5.911)	0.005**

Notes:

Abbreviations: NAC, Neoadjuvant chemotherapy.

* $P < 0.05$, ** $P < 0.01$ are considered as statistically significant.

Continuous variables are reported as median (interquartile range).

Capnocytophaga were more abundant in the non-NAC group than in the NAC group (Fig. 2). Within each group, the taxon with the highest relative abundance in a single sample was *Pseudomonas* (92.7%) in sample N6 of the NAC group and *Streptococcus* (39.7%) in sample C2 of the non-NAC group. The results obtained for the negative control samples are summarized in the Supplemental File.

Comparison of diversity indices by study group

The NAC group showed significantly lower Chao1 ($P = 0.047$), inverse Simpson ($P = 0.008$), and Shannon indices ($P = 0.005$) than the non-NAC group (Table 2). There was also a difference (*i.e.*, beta diversity) between the NAC and non-NAC groups, as determined using PERMANOVA ($P = 0.047$, permutations = 9,999). Complete data for the three diversity indices are presented in Table S1.

DISCUSSION

The traditional belief that “the normal lung is a sterile organ” persisted until recently, when the hypothesis was refuted by the results of NGS-based microbiome analysis (Dickson *et al.*, 2016; Huffnagle, Dickson & Lukacs, 2017). The lower respiratory tract harbors various microorganisms that can be detected using conventional culture-dependent techniques. Early studies of the microbiota in the human respiratory tract were performed

using specimens from bronchoalveolar lavage, bronchoscopic-protected specimen brushes, endotracheal aspirates, and sputum samples. Sputum is universally used for microbial analysis in laboratories but is typically not collected from healthy individuals without respiratory diseases ([Dickson et al., 2014](#)). In this study, we used ETT specimens for microbiome analysis. The ETT contacts the tracheal wall, thereby collecting microorganisms that dwell in the upper part of the lower respiratory tract. The ETT is then extubated *via* the oropharynx and mouth, which are the same tracts where sputum is normally extracted from the lungs. Therefore, we evaluated the ETT as a reliable specimen representing the tracheoropharyngeal microbiome. ETT has not been widely used to analyze healthy respiratory microbiomes ([Cho et al., 2021](#)), mainly because intubation is more methodologically invasive than is collecting other specimens. Nonetheless, for patients undergoing surgery under general anesthesia, collecting ETT data is a simple and reliable means of normal lung microbiota analysis.

Previous studies demonstrated *via* NGS analysis that bacterial communities are abundant in the normal lower respiratory tract. These studies identified *Prevotella*, *Veillonella*, and *Streptococcus* as the main normal flora in the lungs ([Dickson et al., 2015, 2014](#); [Yu et al., 2016](#)). In this study, microbiome diversity was lower in the NAC group than in the non-NAC group.

Healthy lungs possess a rich and diverse microbiome with a low bacterial population ([Faner et al., 2017](#)). Previous studies suggested that normal flora protect the lungs from external pathogens ([Magalhaes et al., 2016](#)), strengthen the immune system ([Vatanen et al., 2016](#)), and improve nutrient uptake. Our data suggest that NAC causes changes in the respiratory microbiota ([Blanton et al., 2016](#)). Previous studies showed that various lung diseases are associated with the dominance of a single taxon, a small group of taxa, or a loss of bacterial diversity ([Tunney et al., 2013](#)). Recent research expanded this knowledge by revealing dysbiosis of the lung microbiome in respiratory diseases such as chronic obstructive pulmonary disease, bronchiectasis, respiratory viral infections, asthma, and even lung cancer ([Yagi et al., 2021](#)).

Lung impairment is a well-known adverse effect of chemotherapy and can range from acute to subclinical modifications ([Taghian et al., 2001](#); [Wong & Evans, 2017](#)). The mechanisms causing lung damage after chemotherapy have been suggested to be structural disruption of the airway system, immune defects, increased inflammatory cells and interleukins in the alveoli ([Bhalla et al., 2000](#); [Wong & Evans, 2017](#)) or a systemic inflammatory response ([Endo et al., 2004](#)). Furthermore, [Liu et al. \(2021\)](#) suggested that alterations in the gut microbiota are related to pneumonia after chemotherapy. Based on the findings of previous studies, microbiome changes in the respiratory tract following chemotherapy may be associated with an increase in pneumonitis or pneumonia; further studies are needed to investigate the relationship.

Based on our findings, decreases in several taxa, such as *Actinomyces*, *Centipea*, and *Moryella*, were more prominent in the NAC group than in the non-NAC group. Therefore, further studies involving monitoring of respiratory microbiomes, including the above taxa, may improve the understanding of the microbial effects and side effects of NAC.

Compared to the non-NAC group, the NAC group had more advanced disease. A recent advance in NGS-based microbiome analysis included the discovery of a link between the human microbiome and diseases. Studies have emphasized that dysbiosis affects cancer development (*Chen et al., 2019*). Numerous studies have been conducted on the gut or breast tumor microbiome, and there is growing evidence suggesting that dysbiosis of the normal flora is associated with breast cancer stage. *Xuan et al. (2014)* indicated that a reduced bacterial load in the tumor tissue is associated with a higher breast cancer stage. Another study by Meng et al. stratified patients with breast cancer by histological grades and showed that grade III tissues had higher alpha diversity than grade I and II tissues (*Meng et al., 2018*). However, in our research conducted with ETT as the respiratory sample, we observed no significant difference in the diversity of indices according to the clinical stage in both NAC and non-NAC groups ([Supplemental file](#)). As this is the first investigation of the trachea-oropharynx microbiome in patients with breast cancer, whether the clinical stage of breast cancer affects the lower respiratory tract microbiome should be further examined. Although we could not clarify whether there was any biological association between breast cancer stage and respiratory microbial alterations, our results provide methodological guidance for further studies.

In the present study, only one sample showed a dominant taxon which occupied more than 50% of the total microbiota. The relative abundance of *Pseudomonas* in sample N6 was 93%. Nevertheless, no clinical symptoms or radiological findings of pneumonia were observed before or after surgery. As there were no signs or symptoms of a respiratory infection, sputum cultures were not performed. Thus, the presence of overdominant taxa in the 16S rRNA amplicon sequencing of the respiratory sample may not directly indicate the presence of bacterial pneumonia.

CONCLUSIONS

We provided initial evidence on the effect of NAC on the tracheo-pharyngeal microbiome of patients with breast cancer. Our results may be used as control data in lung microbiome research of breast neoplasm entities and chemotherapy.

ABBREVIATIONS

NAC	Neoadjuvant chemotherapy
ETT	Endotracheal tube
NGS	Next-generation sequencing

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was supported by the Chung-Ang University Research Grants in 2023. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Chung-Ang University Research Grants in 2023.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Hee Yeon Kim conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Tae Hyun Kim performed the experiments, prepared figures and/or tables, and approved the final draft.
- Jeong Hwan Shin performed the experiments, prepared figures and/or tables, and approved the final draft.
- Kwangrae Cho performed the experiments, prepared figures and/or tables, and approved the final draft.
- Heon-Kyun Ha performed the experiments, prepared figures and/or tables, and approved the final draft.
- Anbok Lee conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Young Jin Kim conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

This study received approval and ethical clearance from the Medical Ethics Committee of the Inje University Busan Paik Hospital (IRB # BPIRB 2019-01-112-003) and written consent was obtained from every participant enrolled.

Data Availability

The following information was supplied regarding data availability:

The sequencing data are available at GenBank: [PRJNA834839](https://www.ncbi.nlm.nih.gov/genbank/PRJNA834839).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.16366#supplemental-information>.

REFERENCES

Adloff C, Andreev V, Andrieu B, Anthonis T, Astvatsatourov A, Babaev A, Bahr J, Baranov P, Barrelet E, Bartel W, Baumgartner S, Becker J, Beckingham M, Beglarian A, Behnke O, Belousov A, Berger C, Berndt T, Bizot JC, Bohme J, Boudry V, Braunschweig W, Brisson V, Broker HB, Brown DP, Bruncko D, Busser FW, Bunyatyan A, Burrage A, Buschhorn G, Bystritskaya L, Campbell AJ, Cao J, Caron S, Cassol-Brunner F, Chekelian V, Clarke D, Collard C, Contreras JG, Coppens YR, Coughlan JA, Cousinou MC, Cox BE, Cozzika G,

- Cvach J, Dainton JB, Dau WD, Daum K, Davidsson M, Delcourt B, Delerue N, Demirchyan R, De Roeck A, De Wolf EA, Diaconu C, Dingfelder J, Dixon P, Dodonov V, Dowell JD, Dubak A, Duprel C, Eckerlin G, Eckstein D, Efremenko V, Egli S, Eichler R, Eisele F, Eisenhandler E, Ellerbrock M, Elsen E, Erdmann M, Erdmann W, Faulkner PJW, Favart L, Fedotov A, Felst R, Ferencei J, Ferron S, Fleischer M, Fleischmann P, Fleming YH, Flucke G, Flugge G, Fomenko A, Foresti I, Formanek J, Franke G, Frising G, Gabathuler E, Gabathuler K, Garvey J, Gassner J, Gayler J, Gerhards R, Gerlich C, Ghazaryan S, Goerlich L, Gogitidze N, Grab C, Grabski V, Grassler H, Greenshaw T, Grindhammer G, Haidt D, Hajduk L, Haller J, Heinemann B, Heinzlmann G, Henderson RCW, Hengstmann S, Henschel H, Henshaw O, Heremans R, Herrera G, Herynek I, Hildebrandt M, Hilgers M, Hiller KH, Hladky J, Hoting P, Hoffmann D, Horisberger R, Hovhannisyanyan A, Ibbotson M, Issever C, Jacquet M, Jaffre M, Janauschek L, Janssen X, Jemanov V, Jonsson L, Johnson C, Johnson DP, Jones MAS, Jung H, Kant D, Kapichine M, Karlsson M, Karschnick O, Katzy J, Keil F, Keller N, Kennedy J, Kenyon IR, Kiesling C, Kjellberg P, Klein M, Kleinwort C, Kluge T, Knies G, Koblitz B, Kolya SD, Korbel V, Kostka P, Koutouev R, Koutov A, Kroseberg J, Kruger K, Kuhr T, Lamb D, Landon MPJ, Lange W, Lastovicka T, Laycock P, Lebailly E, Lebedev A, Leissner B, Lemrani R, Lendermann V, Levonian S, List B, Lobodzinska E, Lobodzinski B, Loginov A, Laktionova N, Lubimov V, Luders S, Luke D, Lytkin L, Malden N, Malinovski E, Mangano S, Marage P, Marks J, Marshall R, Martyn HU, Martyniak J, Maxfield SJ, Meer D, Mehta A, Meier K, Meyer AB, Meyer H, Meyer J, Michine S, Mikocki S, Milstead D, Mohrdieck S, Mondragon MN, Moreau F, et al. 2003. Measurement and QCD analysis of neutral and charged current cross sections at HERA. *The European Physical Journal C* 30(1):1–32 DOI 10.1140/epjc/s2003-01257-6.
- Bhalla KS, Wilczynski SW, Abushamaa AM, Petros WP, McDonald CS, Loftis JS, Chao NJ, Vredenburgh JJ, Folz RJ. 2000. Pulmonary toxicity of induction chemotherapy prior to standard or high-dose chemotherapy with autologous hematopoietic support. *American Journal of Respiratory and Critical Care Medicine* 161(1):17–25 DOI 10.1164/ajrccm.161.1.9903059.
- Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan YM, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta K, Newgard CB, Ashorn P, Dewey KG, Gordon JI. 2016. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science* 351:aad3311 DOI 10.1126/science.aad3311.
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7):581–583 DOI 10.1038/nmeth.3869.
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10(1):421 DOI 10.1186/1471-2105-10-421.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5):335–336 DOI 10.1038/nmeth.f.303.
- Chapadgaonkar SS, Bajpai SS, Godbole MS. 2023. Gut microbiome influences incidence and outcomes of breast cancer by regulating levels and activity of steroid hormones in women. *Cancer Reports* 13:e1847 DOI 10.1002/cnr.2.1847.

- Chen J, Douglass J, Prasath V, Neace M, Atrchian S, Manjili MH, Shokouhi S, Habibi M. 2019. The microbiome and breast cancer: a review. *Breast Cancer Research and Treatment* 178(3):493–496 DOI 10.1007/s10549-019-05407-5.
- Cho SY, Choi JH, Lee SH, Choi YS, Hwang SW, Kim YJ. 2021. Metataxonomic investigation of the microbial community in the trachea and oropharynx of healthy controls and diabetic patients using endotracheal tubes. *PLOS ONE* 16(11):e0259596 DOI 10.1371/journal.pone.0259596.
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation in human body habitats across space and time. *Science* 326(5960):1694–1697 DOI 10.1126/science.1177486.
- Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Beck JM, Huffnagle GB, Curtis JL. 2015. Spatial variation in the healthy human lung microbiome and the adapted Island model of lung biogeography. *Annals of the American Thoracic Society* 12(6):821–830 DOI 10.1513/AnnalsATS.201501-029OC.
- Dickson RP, Erb-Downward JR, Freeman CM, Walker N, Scales BS, Beck JM, Martinez FJ, Curtis JL, Lama VN, Huffnagle GB. 2014. Changes in the lung microbiome following lung transplantation include the emergence of two distinct *Pseudomonas* species with distinct clinical associations. *PLOS ONE* 9(5):e97214 DOI 10.1371/journal.pone.0097214.
- Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. 2016. The microbiome and the respiratory tract. *Annual Review of Physiology* 78(1):481–504 DOI 10.1146/annurev-physiol-021115-105238.
- Dixon P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14:927–930 DOI 10.1658/1100-9233(2003)014[0927:Vaporf]2.0.Co;2.
- Endo S, Sato Y, Hasegawa T, Tetsuka K, Otani S, Saito N, Tezuka Y, Sohara Y. 2004. Preoperative chemotherapy increases cytokine production after lung cancer surgery. *European Journal of Cardio-Thoracic Surgery* 26(4):787–791 DOI 10.1016/j.ejcts.2004.07.015.
- Faner R, Sibila O, Agusti A, Bernasconi E, Chalmers JD, Huffnagle GB, Manichanh C, Molyneaux PL, Paredes R, Perez Brocal V, Ponomarenko J, Sethi S, Dorca J, Monso E. 2017. The microbiome in respiratory medicine: current challenges and future perspectives. *European Respiratory Journal* 49(4):1602086 DOI 10.1183/13993003.02086-2016.
- Garza DR, Dutilh BE. 2015. From cultured to uncultured genome sequences: metagenomics and modeling microbial ecosystems. *Cellular and Molecular Life Sciences* 72(22):4287–4308 DOI 10.1007/s00018-015-2004-1.
- Huffnagle GB, Dickson RP, Lukacs NW. 2017. The respiratory tract microbiome and lung inflammation: a two-way street. *Mucosal Immunology* 10(2):299–306 DOI 10.1038/mi.2016.108.
- Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Molina DA, Salcedo R, Back T, Cramer S, Dai RM, Kiu H, Cardone M, Naik S, Patri AK, Wang E, Marincola FM, Frank KM, Belkaid Y, Trinchieri G, Goldszmid RS. 2013. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342(6161):967–970 DOI 10.1126/science.1240527.
- Kumpitsch C, Koskinen K, Schopf V, Moissl-Eichinger C. 2019. The microbiome of the upper respiratory tract in health and disease. *BMC Biology* 17(1):87 DOI 10.1186/s12915-019-0703-z.
- Liu X, Zou Y, Zhang Y, Liu L, Duan Y, Zhang A, Zhang X, Zhang R, Zhao B, Li X, Wei T, He H, Gan Y, Wang K, Zhu X. 2021. Characteristics in gut microbiome is associated with chemotherapy-induced pneumonia in pediatric acute lymphoblastic leukemia. *BMC Cancer* 21(1):1190 DOI 10.1186/s12885-021-08917-y.

- Magalhaes AP, Azevedo NF, Pereira MO, Lopes SP. 2016.** The cystic fibrosis microbiome in an ecological perspective and its impact in antibiotic therapy. *Applied Microbiology and Biotechnology* **100**(3):1163–1181 DOI [10.1007/s00253-015-7177-x](https://doi.org/10.1007/s00253-015-7177-x).
- Mammen MJ, Sethi S. 2016.** COPD and the microbiome. *Respirology* **21**(4):590–599 DOI [10.1111/resp.12732](https://doi.org/10.1111/resp.12732).
- Martin M. 2011.** Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal* **17**(1):3 DOI [10.14806/ej.17.1.200](https://doi.org/10.14806/ej.17.1.200).
- Meng S, Chen B, Yang JJ, Wang JW, Zhu DQ, Meng QS, Zhang L. 2018.** Study of microbiomes in aseptically collected samples of human breast tissue using needle biopsy and the potential role of in situ tissue microbiomes for promoting malignancy. *Frontiers in Oncology* **8**:318 DOI [10.3389/fonc.2018.00318](https://doi.org/10.3389/fonc.2018.00318).
- Nadeem SO, Jajja MR, Maxwell DW, Pouch SM, Sarmiento JM. 2021.** Neoadjuvant chemotherapy for pancreatic cancer and changes in the biliary microbiome. *American Journal of Surgery* **222**(1):3–7 DOI [10.1016/j.amjsurg.2020.09.042](https://doi.org/10.1016/j.amjsurg.2020.09.042).
- Proc P, Szczepanska J, Zarzycka B, Szybka M, Borowiec M, Ploszaj T, Fendler W, Chrzanowski J, Zubowska M, Stolarska M, Mlynarski W. 2022.** Evaluation of changes to the oral microbiome based on 16S rRNA sequencing among children treated for cancer. *Cancers* **14**:7 DOI [10.3390/cancers14010007](https://doi.org/10.3390/cancers14010007).
- Rubovszky G, Horvath Z. 2017.** Recent advances in the neoadjuvant treatment of breast cancer. *Journal of Breast Cancer* **20**(2):119–131 DOI [10.4048/jbc.2017.20.2.119](https://doi.org/10.4048/jbc.2017.20.2.119).
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011.** Metagenomic biomarker discovery and explanation. *Genome Biology* **12**(6):R60 DOI [10.1186/gb-2011-12-6-r60](https://doi.org/10.1186/gb-2011-12-6-r60).
- Taghian AG, Assaad SI, Niemierko A, Kuter I, Younger J, Schoenthaler R, Roche M, Powell SN. 2001.** Risk of pneumonitis in breast cancer patients treated with radiation therapy and combination chemotherapy with paclitaxel. *JNCI: Journal of the National Cancer Institute* **93**(23):1806–1811 DOI [10.1093/jnci/93.23.1806](https://doi.org/10.1093/jnci/93.23.1806).
- Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C, Ennis M, Boucher RC, Wolfgang MC, Elborn JS. 2013.** Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. *American Journal of Respiratory and Critical Care Medicine* **187**(10):1118–1126 DOI [10.1164/rccm.201210-1937OC](https://doi.org/10.1164/rccm.201210-1937OC).
- Vatanen T, Kostic AD, d’Hennezel E, Siljander H, Franzosa EA, YassouR M, Kolde R, Vlamakis H, Arthur TD, Hamalainen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lahdesmaki H, Huttenhower C, Gevers D, Cullen TW, Knip M, Group DS, Xavier RJ. 2016.** Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* **165**:842–853 DOI [10.1016/j.cell.2016.04.007](https://doi.org/10.1016/j.cell.2016.04.007).
- Wong JL, Evans SE. 2017.** Bacterial pneumonia in patients with cancer: novel risk factors and management. *Clinics in Chest Medicine* **38**(2):263–277 DOI [10.1016/j.ccm.2016.12.005](https://doi.org/10.1016/j.ccm.2016.12.005).
- World Health Organization (WHO). 2020.** Breast cancer 2020 (updated 26 March 2021; cited 2022 14 Jan 2022). Available at <https://www.who.int/news-room/fact-sheets/detail/breast-cancer>.
- Wu AH, Vigen C, Tseng C, Garcia AA, Spicer D. 2022.** Effect of chemotherapy on the gut microbiome of breast cancer patients during the first year of treatment. *Breast Cancer-Targets and Therapy* **14**:433–451 DOI [10.2147/BCTT.S305486](https://doi.org/10.2147/BCTT.S305486).
- Xuan CY, Shamonki JM, Chung A, DiNome ML, Chung M, Sieling PA, Lee DJ. 2014.** Microbial dysbiosis is associated with human breast cancer. *PLOS One* **9**(1):e83744 DOI [10.1371/journal.pone.0083744](https://doi.org/10.1371/journal.pone.0083744).

- Yagi K, Huffnagle GB, Lukacs NW, Asai N. 2021.** The lung microbiome during health and disease. *International Journal of Molecular Sciences* **22(19)**:10872 DOI [10.3390/ijms221910872](https://doi.org/10.3390/ijms221910872).
- Yu G, Gail MH, Consonni D, Carugno M, Humphrys M, Pesatori AC, Caporaso NE, Goedert JJ, Ravel J, Landi MT. 2016.** Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. *Genome Biology* **17(1)**:163 DOI [10.1186/s13059-016-1021-1](https://doi.org/10.1186/s13059-016-1021-1).