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Distribution of *Helicobacter pylori* in the Oral Cavity of Patients With Tooth Extractions at Various Locations and Ages

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ABSTRACT

Background: Helicobacter pylori is well known to cause gastric cancer, gastric ulcers and duodenal ulcers. The oral cavity has been implicated as a reservoir of *H. pylori* in adulthood. However, there are currently no detailed studies focused on the tooth type colonised by *H. pylori* and/or the age of subjects. Therefore, we examined the distribution of *H. pylori* in oral specimens collected from 163 Japanese adults who underwent tooth extraction.

Materials and Methods: Bacterial DNA was extracted from 163 Japanese adults, and *H. pylori* was detected using PCR. Surface deposits on the occlusal surface were observed using an electron microscope.

Results: A PCR analysis revealed *H. pylori* in 17 saliva samples (10.4%), 27 tooth samples (16.6%) and 19 pulp samples (11.7%). We also confirmed surface deposits on the occlusal surface of deeply impacted and obviously stained third molars using electron microscopic images, and *H. pylori* was detected in 11 out of 33 (33.3%) extracted impacted third molars. We then categorised tooth extraction sites into 3 patterns: teeth other than the third molars, erupted third molars and impacted third molars, and compared the ages of subjects who tested positive and negative for *H. pylori*. Among impacted third molars, *H. pylori*-positive subjects were significantly older than *H. pylori*-negative subjects for all oral samples tested ($P < .05$). We then examined these subjects based on the localisation of *H. pylori* in saliva, teeth and dental pulp, and found that subjects with teeth that were positive for *H. pylori* were significantly older than those with teeth that were negative for *H. pylori* ($P < .05$).

Conclusions: H. pylori may colonise not only erupted teeth, but also impacted teeth, particularly in the elderly. Impacted third molars left for a long period may be a reservoir for *H. pylori*.

Introduction

Helicobacter pylori is a Gram-negative microaerophilic bacterium that exhibits strong urease activity and has a helical structure. It is responsible for chronic gastritis, peptic ulcers and gastric cancer.[1] A previous study reported that the prevalence of infection was 8.9% among 1-year-old children, 36.4% among 2-year-old children and then plateaued.[2] Therefore, the acquisition of *H. pylori* infection in a high-risk population appears to mainly occur within the first 2 years of life.[2] The number of new infections decreased markedly after age 2, suggesting that preventive measures such as vaccination would need to be applied very early in infancy.[2] The global prevalence of *H. pylori* among adults has been declining, with

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large regional differences reported, ranging from 24.4% in Oceania to 79.1% in Africa.[3]

Intrafamilial spread is recognised to be the most probable pathway for the transmission of *H. pylori* infection. In intrafamilial *H. pylori* infections, mother-to-child and sibling infections are dominant.[4-6] *H. pylori* bacterial DNA has been detected in dental plaque specimens and saliva, suggesting that the oral cavity may serve as a reservoir and potential source of transmission, particularly in patients with gastric infection.[7-9] *H. pylori* is detected in both the oral cavity and the stomach.[10] However, the number of *H. pylori* in the oral cavity is lower than in the stomach.[10] The oral and gastric environment is influenced by saliva and digested food, and the oral flora is the dominant source of gastric microbiota and therefore a source of *H. pylori* infection and transmission.[10-12] *H. pylori* reservoirs in the oral cavity may include dental plaque, dental caries and saliva. Although the identification of *H. pylori* using tested primer sets has good sensitivity and/or specificity, detection rates for the oral cavity have varied from 0% to 100%[9]; therefore, the role of the oral cavity as a reservoir for *H. pylori* remains controversial.[13] Difficulties in estimating the true infection rate of *H. pylori* within the oral cavity, mainly due to the wide variation in detection rates among studies, have hindered research on transmission and infection sources.

In our previous studies, we described a new method for a nested polymerase chain reaction (PCR) with higher sensitivity than conventional single PCR using a reliable primer set designed based on the genome sequences of approximately 50 *H. pylori* strains.[14-16] When pulp tissue was collected from the same tooth on a different day during root canal treatment, most of the samples that were positive for *H. pylori* in the first sample were also positive for *H. pylori* in the second sample, indicating that *H. pylori* was not present temporarily but had colonised the pulp tissue for a certain period of time.[16] Sequence analysis of nested PCR products was performed in the previous study, confirming that all the amplicons matched the gene sequences of *H. pylori*, thereby supporting the specificity of detection.[16] However, because the interval between the first and second sample collection was approximately 1 to 2 weeks for most subjects, the long-term duration of *H. pylori* colonisation is unknown and further studies are needed to clarify this point.[16] In addition, we attempted to detect *H. pylori* from extracted teeth and saliva using our novel PCR system, and reported a relationship between the presence of *H. pylori* and being overweight.[17] We also suggested that the presence of some oral bacterial species in the oral cavity increases the risk of colonisation by *H. pylori*.[18]

Indications for tooth extraction, including impacted third molars, are non-restorable caries, fracture, infection, periodontal disease, repeated pericoronitis, cysts and tumours.[19] However, the sites at which *H. pylori* is detected in the oral cavity, particularly the third molars, have not yet been investigated in detail. Many adults have impacted third molars, which are often difficult to clean due to their position. This limited accessibility may facilitate bacterial colonisation, making these teeth potential reservoirs for *H. pylori*. Furthermore, it is known that impacted third molars tend to remain in the mouth for long periods with age, and age itself may be a clinical factor involved in the formation of a reservoir for *H. pylori*. Therefore, we examined the relationships between oral colonisation by *H. pylori* and related clinical factors, with a focus on the impacted third molars.

Materials and method

Ethics statement

The present study was conducted in full adherence to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Osaka University Graduate School of Dentistry (approval no. H27-E17, H30-E32). Prior to specimen collection, all subjects were informed of the study protocol and provided their written informed consent.

Subjects

Saliva and extracted teeth were obtained from 163 Japanese adults (79 males and 84 females, age range: 16-83 years; median: 39.0 years; mean age: 41.7 ± 19.5 years) who had dental issues that required tooth extraction, such as dental caries, periodontitis and pericoronitis, and received a referral to the Department of Oral and Maxillofacial Surgery at Osaka University Dental Hospital between March 2016 and June 2021. We collected approximately 1 mL of saliva before tooth extraction. Then, we performed the tooth extraction and placed the extracted teeth into 2.5 mL of sterile saline in a sterile disposable tube. Both saliva samples and extracted teeth were stored at −20°C immediately after collection and were tested within 1 month. Saliva and extracted teeth were then analysed separately. Oral hygiene habits and systemic *H. pylori* infection status (eg, urea breath test or gastric biopsy) were not assessed in this study.

Oral cavity specimens

Dental plaque was collected from all extracted teeth. Sonication was used to separate the dental plaque from the 2.5 mL of sterile saline containing the extracted teeth.[18] After the tooth removal, the suspension was centrifuged, and the supernatant was discarded. The resulting suspension served as a dental plaque specimen from the extracted tooth. Next, the pulp chamber of the extracted tooth was opened using a sterilised dental hand piece and diamond point, and the dental pulp specimen was deposited into a sterile plastic tube containing 1 mL of sterile saline.[15] Saliva (1 mL) from each subject was collected into a sterile disposable tube. The dental pulp and saliva specimens were centrifuged, and the supernatant was discarded.

Molecular biological analysis

Following the extraction of bacterial DNA from saliva, pulp and extracted teeth (dental plaque), it was resuspended in 100 μL distilled water.[15] Bacterial DNA was then subjected to nested PCR using primer sets, which produced amplicons consistent in size for genomic DNA of all *H. pylori* strains, but were not seen in *Helicobacter pullorum* and *Helicobacter felis* strains.[16] In brief, first-step PCR was conducted on 2 μL

bacterial DNA in reactions with a total volume of 20 μL using the primers *ureA*-aF (5′-ATG AAA CTC ACC CCA AAA GA-3′) and *ureA*-bR (5′- CCG AAA GTT TTT TCT CTG TCA AAG TCT A-3′). One microliter of the first-PCR product was then used in second-step PCR as a template in reactions with a total volume of 20 μL using the primers *ureA*-bF (5′-AAA CGC AAA GAA AAA GGC ATT AA-3′) and *ureA*-aR (5′-TTC ACT TCA AAG AAA TGG AAG TGT GA-3′). First- and second-step PCR amplifications were conducted using *TaKaRa Ex Taq* (Takara Bio. Inc.) with the following cycling parameters: initial denaturation at 95°C for 4 minutes followed by 30 cycles at 95°C for 30 seconds, at 55°C for 30 seconds and at 72°C for 30 seconds, with the final extension at 72°C for 7 minutes. PCR products were then subjected to electrophoresis on a 1.5% agarose gel.

Electron microscopy observations

The following steps were performed to prepare samples for scanning electron microscopy (SEM)[20]: washing, fixation with 2% osmium tetroxide and 1% glutaraldehyde, dehydration with ethanol, and drying with *t*-butyl alcohol using the freeze-drying method. Dried samples were then mounted on the stage of the electron microscope, coated with osmium for conductive processing, and examined under SEM. Field-emission SEM (FE-SEM; Hitachi S-4800) was performed to identify sample morphologies.

Sequence analysis

Sequencing of the V1 to V2 (V12) region was performed using bacterial DNA.[21,22] V12 amplicons were prepared using the forward primer (16S_ 27Fmod: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AGR GTT TGA TYM TGG CTC AG) and reverse primer (16S_338R: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTG CTG CCT CCC GTA GGA GT). V3 to V4 amplicons (V34) were prepared using the forward primer (16S_341F: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and reverse primer (16S_805R: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C) with the KAPA HiFi HotStart Ready Mix (Roche, Basel, CHE). Sequencing libraries were prepared according to the 16S library preparation protocol provided by Illumina. The Nextera XT Index kit (Illumina) was used to attach dual index adapters for sequencing on the Illumina MiSeq platform. Each sequencing library was diluted to 5 ng/μL. Equal volumes of the libraries were mixed to a final concentration of 4 nM. To measure the DNA concentrations of the mixed libraries, a quantitative real-time PCR (qPCR) was performed with the KAPA SYBR FAST qPCR Master mix (Roche) using primer 1 (AAT GAT ACG GCG ACC ACC) and primer 2 (CAA GCA GAA GAC GGC ATA CGA). Mixed libraries were then sequenced in a 250-bp paired-end run for V12 using the MiSeq Reagent Kit v2 (500 cycles) and in a 300-bp paired-end run for V34 using the MiSeq Reagent Kit v3 (600 cycles). The qPCR library quantification described here is intended to quantify library concentration for next-generation sequencing, not *H. pylori* abundance.

Bioinformatic analysis

Raw sequence data were analysed using the QIIME 2 pipeline (version 2020.2) and denoised using DADA2 in the QIIME 2 package[23] according to the following strategy: the construction of an ASV table with paired-end sequencing reads, followed by the assignment of taxonomic information using Greengenes.[24]

Statistical analysis

GraphPad Prism 9 (GraphPad Software Inc.) was used for statistical analyses. The Mann-Whitney U test was performed to assess the significance of intergroup differences between 2 groups. For comparisons among more than 2 groups, the Kruskal-Wallis test and non-parametric analysis for multiple comparisons were used. *P* values < .05 indicated a significant difference.

Results

Distribution of H. pylori *in subjects*

This study focused on the site of tooth extraction. The subjects were 163 Japanese adults who underwent tooth extractions at a single site, and the proportions by tooth extraction site (teeth other than the third molars, erupted third molars, and impacted third molars) are shown (Figure 1A). We detected *H. pylori* in saliva, extracted tooth and pulp samples using our nested PCR method. We divided 163 subjects into 2 groups according to whether any of their oral samples tested negative (n = 113; 69.3%) or positive (n = 50; 30.7%) for *H. pylori*. *H. pylori*-positive subjects were then subdivided into those with *H. pylori* in saliva (n = 17; 10.4%), extracted teeth (n = 27; 16.6%) and dental pulp (n = 19; 11.7%) (Figure 1B). When the detailed distribution of *H. pylori* in each oral specimen was analysed, *H. pylori* was detected in saliva (n = 9; 18.0%), teeth (n = 17; 34.0%), dental pulp (n = 13; 26.0%), saliva and teeth (n = 5; 10.0%), saliva and dental pulp (n = 1; 2.0%), teeth and dental pulp (n = 3; 6.0%), and all samples (n = 2; 4.0%) (Figure 1C). No significant differences were observed in age between the *H. pylori*-positive and -negative groups (Figure 1D).

Evaluation of an impacted mandibular third molar

We focused on a deeply impacted third molar extracted one of the subjects, which had not erupted into the oral cavity (Figure 2A). A panoramic radiographic image revealed an impacted mandibular third molar located near the root apex of the right mandibular second molar (Figure 2B). Once extracted, the occlusal surface of the deeply impacted mandibular third molar showed considerable staining (Figure 2C, D).

Oral microbiome in impacted third molars

Since we confirmed the presence of bacteria in the impacted third molar, we examined panoramic

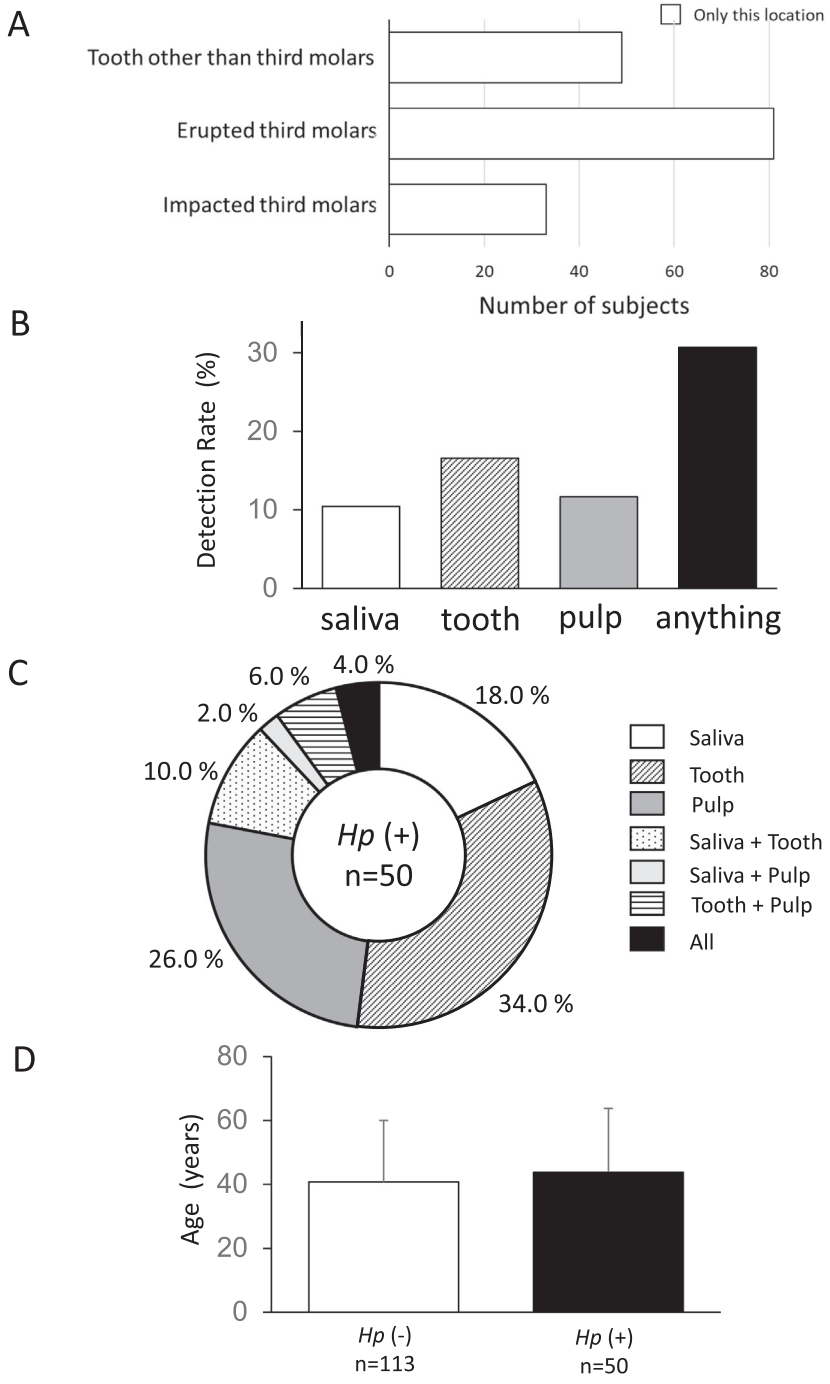

Figure 1 – Detection of *H. pylori* in oral specimens. (A) Breakdown of subjects who participated in this study. (B) Rates of detection of *H. pylori* in oral specimens of saliva, teeth, pulp, and anything. (C) Rates of detection of *H. pylori* in oral specimens. (D) Comparison of age between the *H. pylori*-negative and -positive groups. Data are mean ± S.D.

radiographs of 33 patients who required the extraction of impacted third molars (Figure 3A). *H. pylori* was detected in saliva, teeth, or dental pulp in 11 out of 33 subjects (Figure 3B). Case 11 had 2 impacted teeth extracted, and *H. pylori* was detected in all saliva, tooth and pulp samples; therefore, we performed a detailed examination of the bacterial flora. In a class level analysis, Bacilli was more frequently detected in teeth (range, 41.9%-58.1%) than in pulp (range, 17.9%-24.2%). The relative frequencies for Clostridia (12.6%-23.1%) and Actinobacteria (13.9%-18.2%) were small between samples. The relative frequency of Bacteroidia varied between oral samples: 12.6 and 28.7% in dental pulp and 1.8 and 11.4% in teeth (Figure 3C). No significant difference was observed in the oral microbiome between tooth locations and the presence or absence of *H. pylori*.

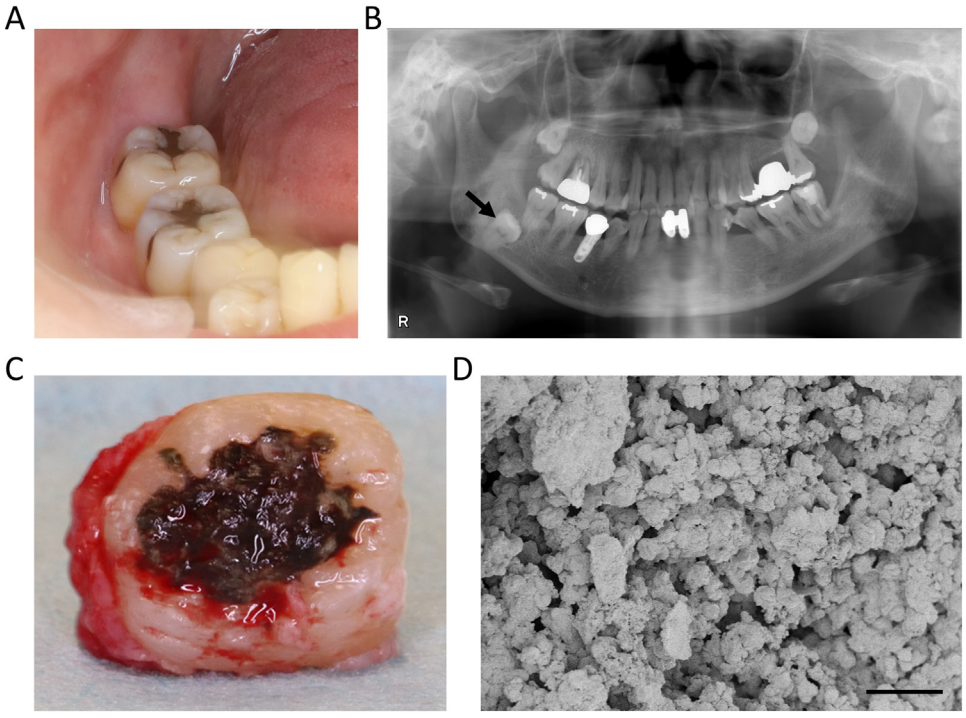

Figure 2 – Analysis of an impacted mandibular third molar. (A) Intraoral photograph taken before extraction. (B) Panoramic radiography taken before extraction revealed an impacted third molar. Arrows indicate the impacted third molar. (C) Extracted tooth. (D) Representative transmission electron micrographs of the extracted tooth. Scale bar, 20 μm.

Distribution of H. pylori *by extraction sites in subjects*

We examined extraction sites in 3 patterns: teeth other than the third molars, erupted third molars and impacted third molars. In 49 subjects who underwent tooth extraction other than the third molars, subjects who tested positive for *H. pylori* were subdivided into those with *H. pylori* in saliva (n = 4; 8.2%), extracted teeth (n = 9; 18.4%) and dental pulp (n = 6; 12.2%) (Figure 4A, B). Among 81 subjects with erupted third molars, those who tested positive for *H. pylori* were subdivided into those with *H. pylori* in saliva (n = 9; 11.1%), extracted teeth (n = 12; 14.8%) and dental pulp (n = 9; 11.1%) (Figure 4C, D). Among 33 subjects with impacted third molars, those who tested positive for *H. pylori* were subdivided into those with *H. pylori* in saliva (n = 4; 12.1%), extracted teeth (n = 6; 18.2%) and dental pulp (n = 4; 12.1%) (Figure 4E, F).

Relationship between the presence or absence of H. pylori *and the age of subjects*

We examined the age of subjects with or without *H. pylori*. In all 163 cases, no significant difference was observed in the detection of *H. pylori* in saliva, tooth, or pulp samples based on age (Figure 5A). However, when assessed by site, subjects with an impacted third molar were older in the *H. pylori*-positive group than in the *H. pylori*-negative group (Figure 5B). Therefore, we investigated the age at which *H. pylori* was detected in saliva, teeth and dental pulp at each site. No significant differences were noted in saliva samples for any tooth type with or without *H. pylori* (Figure 5C). Regarding teeth, the presence and absence of *H. pylori* did not significantly differ in teeth other than the third molars and erupted third molars based on age (Figure 5D). However, subjects with impacted third molars were significantly older in the *H. pylori*-positive group (average 45.3 years) than in the *H. pylori*-negative group (average 28.1 years) (Figure 5D). Furthermore, there was no significant difference in dental pulp samples for any tooth type with or without *H. pylori* (Figure 5E).

Discussion

A previous study reported that the prevalence of infection was 8.9% among 1-year-old children, 36.4% among 2-year-old children and then plateaued.[2] The global prevalence of *H. pylori* among adults has been declining, with large regional differences reported, ranging from 24.4% in Oceania to 79.1% in Africa.[3] The prevalence in Japan among adults is likewise decreasing and is currently estimated at approximately 35%.[25] *H. pylori* bacterial DNA has been detected in dental plaque specimens and saliva,[7,8] *H. pylori* reservoirs in the oral cavity may include dental plaque, dental caries and saliva. However, the role of the oral cavity as a reservoir for *H. pylori* remains controversial.[13] Therefore, we investigated *H. pylori* in Japanese adults who underwent tooth extraction. The results obtained showed that *H. pylori* was present in the oral cavity of 50 out of 163 subjects (30.7%). Subjects who tested positive for *H. pylori* were subdivided into those with *H. pylori* in saliva (n = 17; 10.4%), extracted teeth (n = 27; 16.6%) and dental pulp (n = 19; 11.7%) (Figure 6). Various locations in the

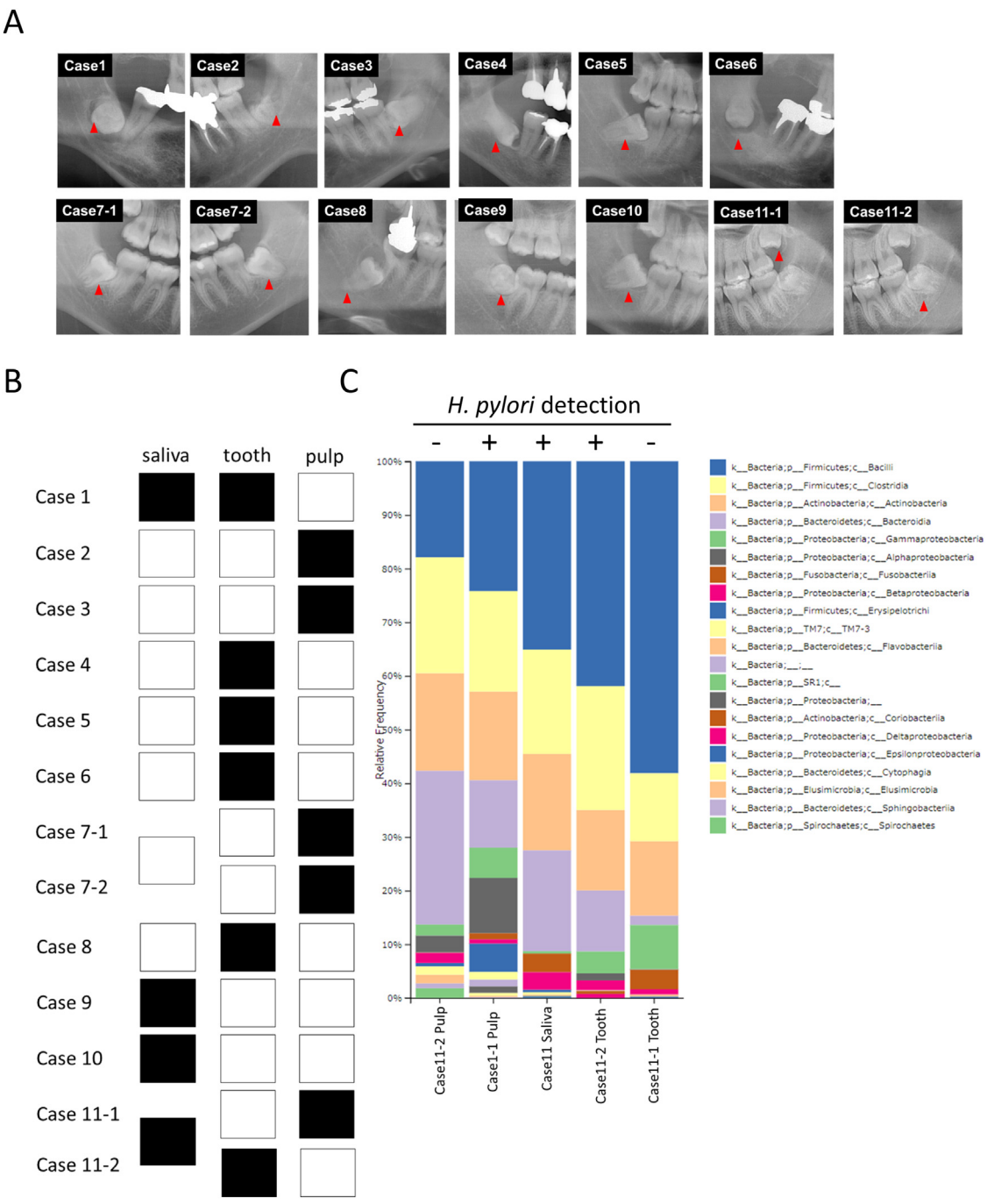

Figure 3 – Bacterial flora in impacted third molars. (A) Panoramic photographs of *H. pylori*-positive subjects with an impacted third molar. (B) Distributions of *H. pylori* in oral samples. Black and white squares indicate the positive and negative detection of *H. pylori*, respectively. (C) The relative abundance of oral bacterial communities in Case 11. (D)

oral cavity, such as saliva, teeth and pulp, may be reservoirs for *H. pylori*.

Third molars contribute little to essential oral functions such as mastication, are often poorly positioned and difficult to clean, and are frequently associated with pain and complications.[26,27] In addition, 65% of healthy individuals have an impacted tooth, which is often difficult to treat due to its position and is frequently extracted.[26,27] Based on these findings, we speculated that third molars may be *H. pylori* reservoirs in the oral cavity. The occlusal surface of a deeply impacted mandibular third molar in one of our subjects, which was not visible in the oral cavity, was found to have considerable staining. We detected structures that appeared to be surface deposits under an electron microscope. *H. pylori* colonisation may involve caries on the occlusal surface, or even if we cannot see the teeth in the oral cavity, bacteria and contaminants can still enter through periodontal pockets or any gaps. Although further histological or *in vitro* adhesion

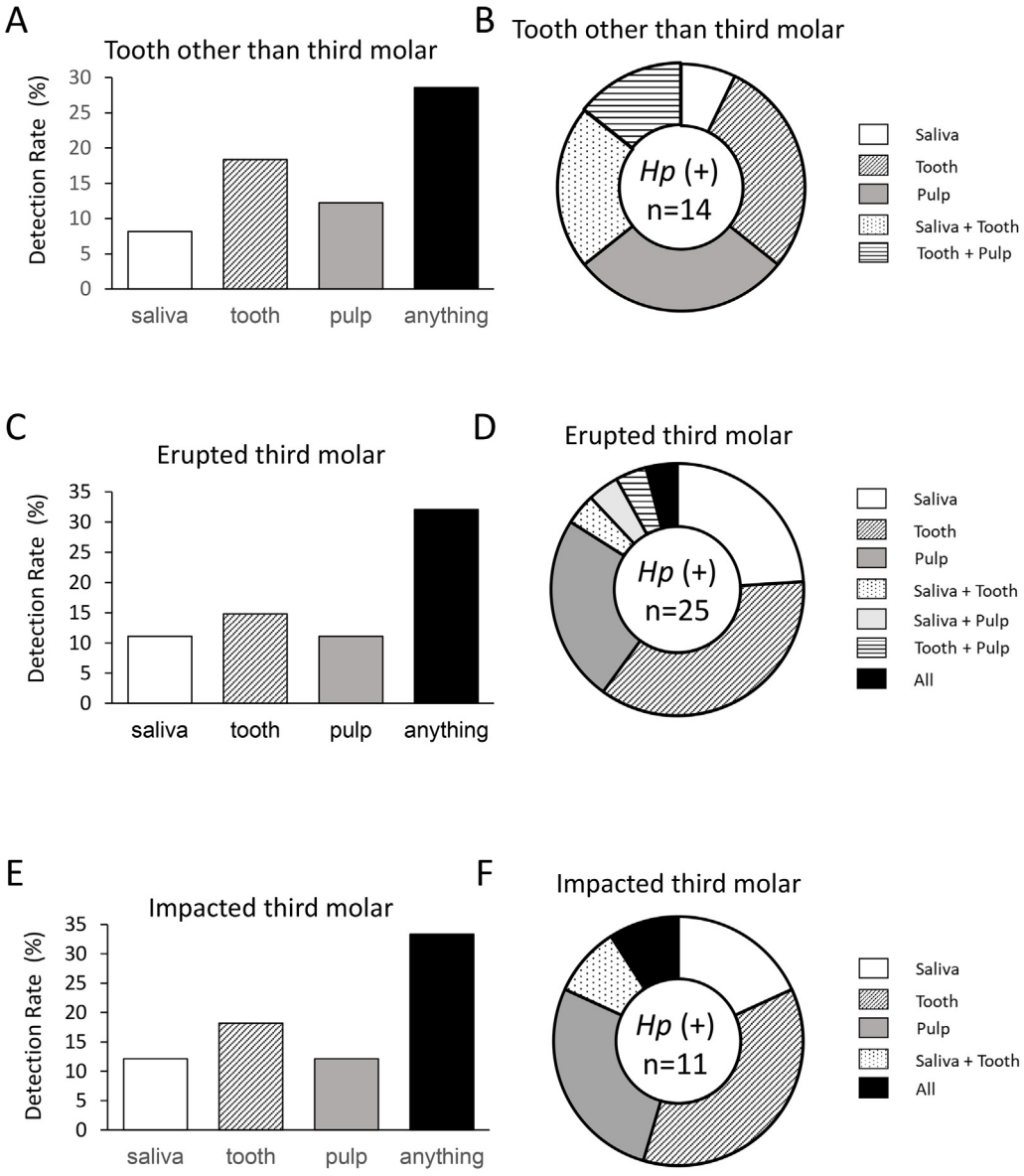

Figure 4 – Distribution of *H. pylori* in different extracted tooth types. Rates of detection of *H. pylori* among oral specimens of saliva, teeth, pulp, and anything in 3 groups: teeth other than the third molars (A, B), erupted third molars (C, D), and impacted third molars (E, F).

assays may be necessary to clarify the mechanism by which *H. pylori* colonise impacted teeth, our results suggest that even fully impacted third molars may be reservoirs for *H. pylori* (Figure 6). Given that third molars are often retained or extracted at different ages, we considered the potential role of age in the colonisation of *H. pylori*. Older individuals are more likely to retain symptomless impacted third molars, which may serve as long-term reservoirs for *H. pylori*.

The majority of studies that have investigated *H. pylori* in the oral cavity focused on dental plaque, extracted tooth, dental pulp, or saliva specimens.[15,17,18,28,29] In the present study, *H. pylori* was detected in the dental pulp of 11.7% of all samples, and in the pulp of impacted teeth, the rate was 12.1%, which was no different from the rate for other teeth. The mechanism of infection may be retrograde or haematogenous infection of the dental pulp, or contamination. In the experimental procedures, we took measures to prevent contamination, such as using a negative control in which sterile purified water (MQ) was added instead of DNA to control for contamination, and using tips with filters. In addition, our previous report showed that *H. pylori* could be detected in dental pulp tissue even in cases where the bacteria was not detected in saliva, suggesting that dental pulp could serve as a reservoir for *H. pylori*.[15] Furthermore, the present study also demonstrated that *H. pylori* was detected in dental pulp even when it was not detected in saliva or teeth, suggesting that the possibility of contamination is considered low.

De Bruyn et al[30] examined 1682 patients (818 males and 864 females, mean age 31 years) to clarify why they retained

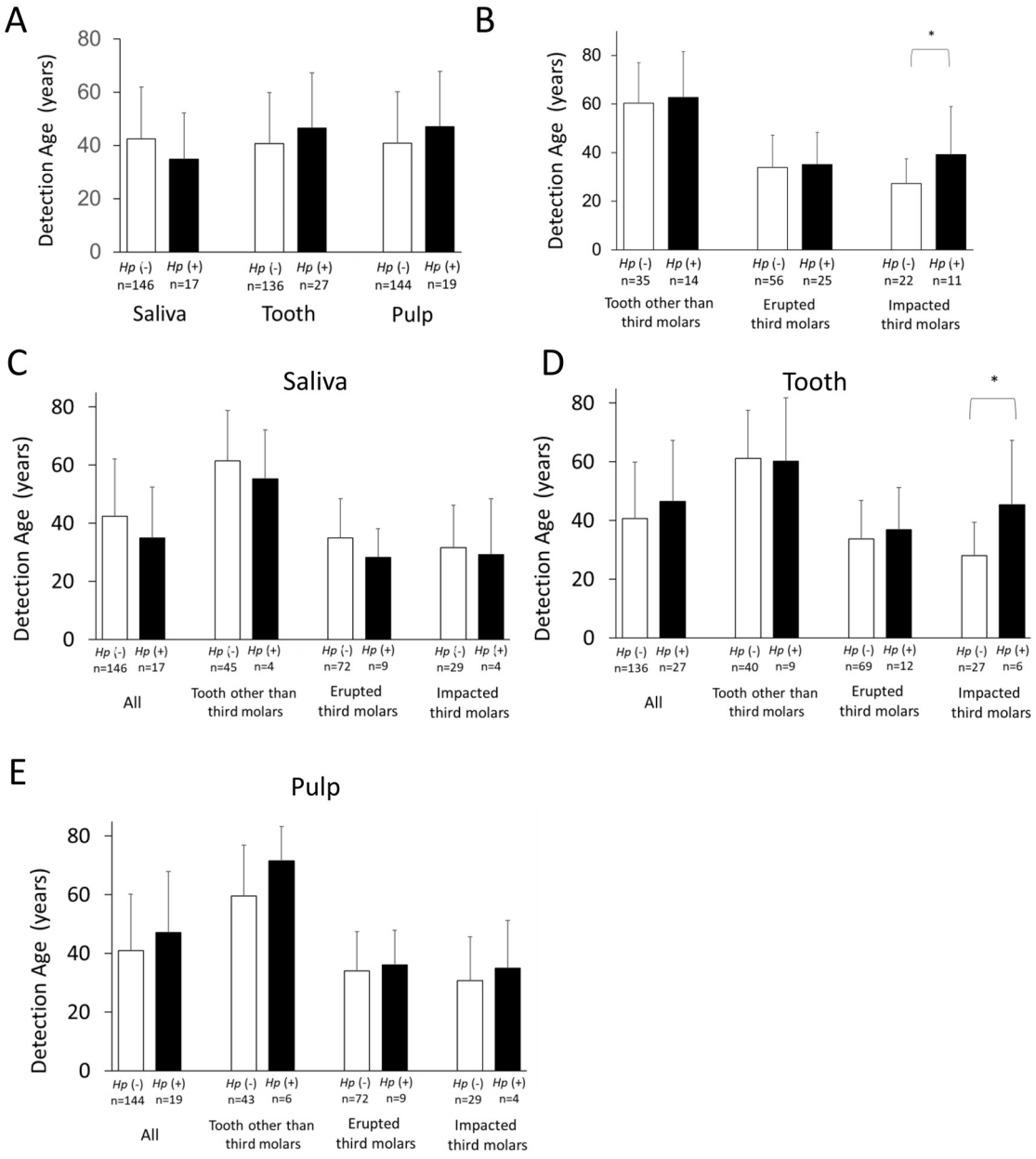

Figure 5 – *H. pylori* detection according to age. (A) Comparison of age between *H. pylori*-negative and -positive groups by saliva, tooth, and pulp for all samples. (B) Comparison of age between *H. pylori*-negative and -positive groups in 3 different tooth types: teeth other than the third molars, erupted third molars, and impacted third molars. Comparison of age between *H. pylori*-negative and -positive groups among saliva (C), teeth (D), and pulp (E) in 3 different tooth types: teeth other than the third molars, erupted third molars, and impacted third molars. Data are mean ± S.D.

their third molars. The most frequent reason was eruption into proper occlusion (31.9%); however, the frequencies of reasons for retaining the third molar differed according to age.[30] At younger ages, patient preference (51.1%) was the most common reason, followed by eruption into proper occlusion (22.2%) and adequate space for eruption (8.6%). In patients in their 30s and 40s, eruption into proper occlusion (41.1%) was the most common reason, followed by symptomless third molars (27.6%) and patient preference (19.6%). In contrast, the most common reasons in patients in their 50s and 60s were eruption into proper occlusion (33.3%), symptomless third molars (33.3%) and patient preference (12.5%), while in those older than 70 years, eruption into proper occlusion (45.9%) was the most common reason.[30] Patient preference was the most common in young patients, while symptomless third molars and eruption into proper occlusion were more common in older patients.[30] In the case of impacted third molars, symptomless molars or potential damage to adjacent structures was a more common reason than eruption into proper occlusion. In Figure 2, the patient

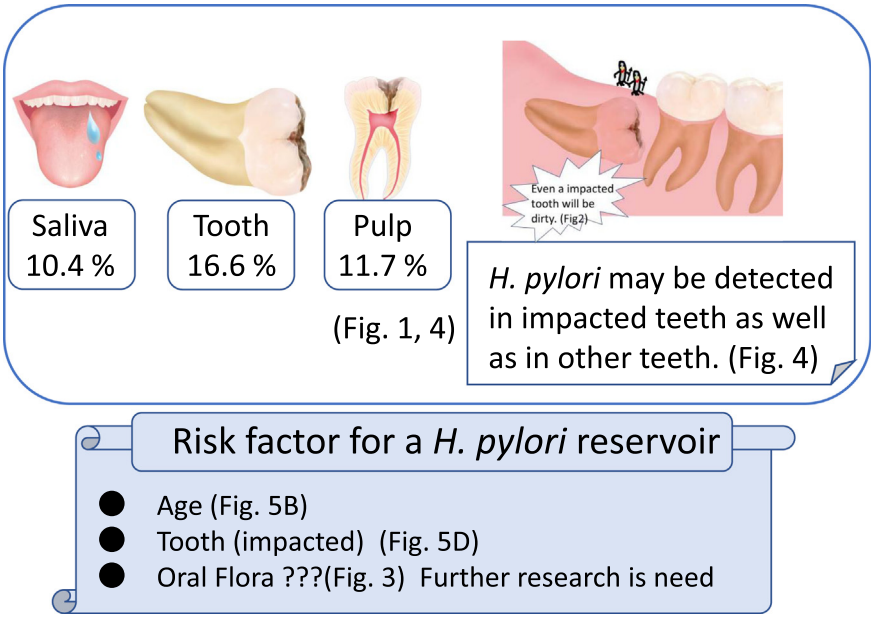

Figure 6 – Schematic representation of the mechanism of *H. pylori* infection in a patient with an impacted mandibular third molar.

had hesitated to undergo tooth extraction because a previous dentist indicated the possibility of a facial incision and the risk of nerve damage. However, the patient decided to have the tooth extracted due to increasing pain and other symptoms. In the present study, the age of subjects differed based on the presence or absence of *H. pylori* only among those with impacted third molars. Therefore, the extraction of an impacted tooth at a younger age may eliminate the risk of *H. pylori* infection. It has been reported that after the extraction of a molar containing *H. pylori*, *H. pylori* was no longer detectable in the oral cavity or by urea breath tests.[31] However, direct evidence, including long-term data, showing an association between tooth extraction and a reduction in *H. pylori* load is currently lacking, and future long-term and comprehensive studies are required to clarify the causal relationship.

Comparisons of the detection rates of *H. pylori* and age for saliva, tooth and pulp samples in the impacted third molar group of patients showed a significant difference in age only. These results suggest that impacted third molars were reservoirs for *H. pylori*, particularly in older subjects (Figure 6). Age-related increases in alveolar bone crest alterations may occur due to impacted third molars.[32] The underlying reasons include the possibility of remaining third molars at an advanced age due to symptomless third molars or potential damage to adjacent structures becoming reservoirs over time.

Even after the eradication of *H. pylori*, a certain recurrence rate has been reported, and recurrence remains a significant global challenging public health issue.[33] This study suggested that impacted third molars may function as a reservoir for *H. pylori* and clarified that age may be involved in this association. These results suggest that the presence of impacted third molars may contribute to the recurrence of *H. pylori*, and screening for impacted third molars may be clinically useful for evaluating the risk of recurrence and for long-term management after eradication, especially in elderly people.

According to the study of Ogaya et al,[34] the oral microbiome of *H. pylori*-positive children was more diverse than that of *H. pylori*-negative children. Their study also demonstrated that, in both adults and children, *Porphyromonas* was more abundant in the *H. pylori*-positive oral microbiome than in the *H. pylori*-negative oral microbiome.[34] These finding collectively indicate that a unique oral microbiome was recently identified in subjects with oral *H. pylori* infection, particularly in children.[34] Furthermore, the oral microbiome varies widely across populations and disease states. For example, differences in subgingival microbiota have been reported between Vietnamese living in Germany and German periodontitis patients.[35] These variations may affect the colonisation and persistence of bacteria like *H. pylori* in the oral cavity. In contrast, in the present study did not find significant association between the presence or absence of *H. pylori* and the oral microbiome among oral specimens from a single subject. Our result may suggest that *H. pylori* colonisation at different oral sites within a single individual is independent of the broader microbiome. However, this study was based on a single case, and there are limitations to generalising the results. Therefore, further investigations involving a larger sample size are necessary to validate and expand these results. Furthermore, most amplicon sequence variants (ASVs) could only be classified at the class level, limiting detailed taxonomic interpretation. In addition, in the future, it will be necessary to study the oral microbiome not only on the same patient as in the present study, but also on each site, such as saliva, tooth and pulp, and also on each extraction site, such as teeth other than the third molars, erupted third molars and impacted third molars. The small number of specimens subjected to a microbiome analysis is a limitation of the present study and,

thus, a detailed analysis of more specimens is required in the future.

This study has some limitations. This study was cross-sectional in design, and it cannot capture changes over time. We will need to plan longitudinal studies focusing on prolonged exposure or immune senescence to propose a mechanism for colony formation associated with aging. In addition, the possibility of contamination is considered low, but it cannot be completely ruled out, and the possibility of false positives or false negatives in PCR must be considered.

In conclusion, the present results indicate the presence of *H. pylori* in deeply impacted as well as erupting teeth. In the present study, age was identified as one of the risk factors for impacted third molars becoming a *H. pylori* reservoir.

Conflict of interest

None disclosed.

Author contributions

M.H. and R.N. designed the study under the supervision of K.N. (Kazuhiko Nakano). M.H. collected clinical samples and R.N., Y. O., K.N. (Kyoko Nishiyama) and T.K. performed experiments. Data interpretation was conducted by M.H., R.N. and K.N. (Kazuhiko Nakano). M.H., R.N. and K.N. (Kazuhiko Nakano) wrote the manuscript, which all authors read and approved.

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Data availability

Data are not available for public access because of patient privacy concerns, but are available from the corresponding author upon reasonable request.

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