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Geographic signatures in the oral resistome: a comparative metagenomic analysis of healthy individuals from Thailand and Norway

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ABSTRACT

Background: The oral cavity is an important yet understudied reservoir of antimicrobial resistance genes (ARGs), potentially shaped by geographic variation in antibiotic usage.
Objective: To compare the oral resistomes of healthy adults from Thailand and Norway, two countries with contrasting antimicrobial use practices, using shotgun metagenomic sequencing.
Design: Stimulated saliva samples were collected from healthy adults in Thailand ($n = 43$) and Norway ($n = 50$). ARGs were identified with AMRPlusPlus against the MEGARes database, and microbial taxonomy was profiled with KrakenUniq. Diversity metrics, ordination, and clustering analyses assessed resistome and microbiome structures.
Results: Thai samples exhibited significantly greater ARG richness, evenness, and diversity ($p < 0.001$), driven by higher abundances of multi-biocide, nucleoside, and copper resistance genes. Norwegian samples were enriched in aminoglycoside, sulfonamide, and quaternary ammonium compound resistance genes. Both cohorts shared core oral genera, but Thai samples showed greater taxonomic richness without differences in overall microbiome diversity. Non-metric multidimensional scaling and PERMANOVA revealed stronger geographic separation for resistomes ($R^2 = 0.639$) than microbiomes ($R^2 = 0.382$). Co-occurrence networks highlighted structured associations between ARG groups and bacterial genera, suggesting ecological influences beyond taxonomic composition.
Conclusions: These results reveal distinct geographic signatures in the oral resistome that are not fully explained by microbiome structure, reflecting the influence of local ecological and societal factors, including antimicrobial exposure. The findings highlight the oral cavity as a dynamic ARG reservoir and support its inclusion in regional antimicrobial resistance surveillance to inform public health strategies.

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Introduction

The human oral cavity harbors one of the most densely colonized and metabolically active microbial ecosystems in the body [1]. Beyond its role in oral health, the oral microbiome influences systemic immune function and metabolic homeostasis, acting as a crucial interface between the host and the external environment [2]. While the taxonomic composition and functional roles of the oral microbiome have been widely studied, increasing attention is now being directed toward its potential as a reservoir of antimicrobial resistance genes (ARGs), collectively known as the resistome, which has been extensively studied in the gut microbiome but remains less explored in the oral niche [3,4].

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The oral resistome comprises both intrinsic resistance genes and those acquired through horizontal gene transfer [5,6]. Several metagenomic studies have identified a wide array of ARGs in the oral cavity, including those conferring resistance to macrolides, tetracyclines, beta–lactams, and biocides, with many predicted to be associated with MGEs [7–12]. Frequent exposure of the oral environment to antimicrobials, via systemic antibiotics, antiseptic dental products (such as mouthwashes or toothpastes containing antimicrobial agents), and over–the–counter medications, may promote the enrichment and maintenance of resistance genes within this niche [13–16]. Moreover, the high microbial density and extensive biofilm formation typical of oral communities facilitate genetic exchange and persistence of resistance elements [17]. These features raise concerns that the oral microbiome may act as a dynamic reservoir of ARGs capable of transferring resistance determinants to transient or pathogenic bacteria, potentially contributing to systemic infections and the wider antimicrobial resistance burden.

Recent developments in shotgun metagenomic sequencing have enabled culture–independent, high–resolution profiling of ARGs across diverse microbial communities. Such approaches have uncovered striking differences in resistome composition across individuals, populations, and geographic regions, often reflecting variations in antibiotic usage patterns, healthcare infrastructure, and regulatory practices. A notable example is the study by Carr et al. [18], which analyzed oral and gut metagenomes from multiple countries and demonstrated site–and region–specific differences in ARG abundance and diversity [18]. However, that study relied on publicly available datasets with variable sample types and protocols, limiting the ability to make direct cross–population comparisons.

Although several studies have examined the oral resistome in individual populations or as part of larger microbiome surveys, systematic cross–country comparisons using harmonized metagenomic approaches remain limited. To address this gap, we performed a standardized, comparative metagenomic analysis of the oral resistome and microbiome in healthy individuals attending routine oral health screening appointments in Thailand and Norway, two countries that exemplify noticeably contrasting antimicrobial use landscapes in their societies. Thailand, like many countries in Southeast Asia, experiences high levels of antibiotic consumption for human use, with 50.9 Defined Daily Doses (DDD) per 1,000 inhabitants per day in 2019 and less stringent regulation [19,20], while Norway represents one of the lowest antibiotic usage contexts globally with 13.6 DDD per 1,000 inhabitants per day in 2019, supported by strong antimicrobial stewardship programs [21,22]. These differences provide a natural contrast to investigate how societal–level antibiotic exposure may shape the oral resistome.

Given that antimicrobial resistance prevalence in the oral niche may be shaped by national prescribing patterns, direct cross–country comparisons can provide critical insights into how stewardship policy differences manifest in commensal microbial communities. In this study, we applied uniform saliva collection, DNA extraction, and shotgun metagenomic sequencing protocols across both cohorts to minimize technical variability. Our primary aim was to characterize and compare the abundance, diversity, and taxonomic associations of ARGs in the oral microbiomes of Thai and Norwegian individuals. By integrating taxonomic and resistome profiles through correlation analysis, we sought to explore the ecological drivers underlying ARG distribution and provide insights into the role of the oral cavity as a dynamic reservoir of antimicrobial resistance.

Materials and methods

Study participants and saliva sample collection

Saliva samples were collected from healthy volunteers aged between 21 and 65 years who had not used antibiotics within the three months prior to saliva sample collection. All participants were healthy adults without self–reported oral or systemic diseases and were recruited during routine oral health visits. Formal dental indices (e.g. DMFT, plaque index) were not recorded; however, all participants reported having no active oral infections or ongoing dental treatments at the time of sampling. The sample size represented all eligible volunteers recruited within the defined study period under harmonized collection and sequencing protocols, ensuring comparability between the two cohorts. Participants were instructed to refrain from eating, drinking, and brushing their teeth for at least one hour prior to sample collection.

Norwegian saliva samples were obtained from individuals attending the University Dental Clinic at UiT The Arctic University of Norway between October and November 2018, while Thai saliva samples were collected from individuals visiting the dental clinic at Thammasat University Hospital between July and October 2020. Ethical approvals were obtained from the Regional Committees for Medical and Health Research Ethics (Project number 2018/1373/REK nord) and the Ethical Review Sub–Committee Board of Human Research Involving Science, Thammasat University, Thailand (COA No. 163/2562) for the collection of Norwegian and Thai saliva samples, respectively. Written informed consents were obtained from all participants prior to the collection of saliva samples.

Saliva secretion was stimulated using sterile paraffin gum, and approximately 2 mL of saliva was collected from each participant using the Saliva DNA Collection and Preservation Kit (Norgen Biotek Corp., Ontario, Canada). All samples were anonymized with IDs starting with 'T' for Thai participants and 'N' for Norwegian participants, followed by numbers, and were stored at room temperature until further processing.

Saliva metagenomic DNA extraction

Saliva metagenomic DNA was extracted using an enzymatic pre–treatment protocol optimized with MetaPolyzyme (Merck, Norway), as established in a prior study [23]. Briefly, 550 µL of each saliva sample was transferred into a 2-mL microcentrifuge tube and mixed with 50 µL of MetaPolyzyme solution. The mixture was incubated at 35 °C for 5 hours to ensure efficient cell lysis of both Gram–positive and Gram–negative bacteria. DNA was subsequently extracted using the QIAamp® DNA Mini Kit on a QIAcube automated platform (Qiagen, Norway), following a modified protocol in which the volumes of proteinase K, Buffer AL, and ethanol were adjusted to 40 µL, 400 µL, and 400 µL, respectively, to enhance yield and purity. The quality and quantity of extracted DNA were assessed by agarose gel electrophoresis for integrity, NanoDrop spectrophotometry (Thermo Fisher Scientific) for purity, and Qubit fluorometric quantification (Thermo Fisher Scientific, MA, USA) for concentration.

Metagenomic sequencing and bioinformatic processing

Library preparation and shotgun metagenomic sequencing were performed by the Beijing Genomics Institute (Shenzhen, China) using the DNBSEQ platform to generate 150 bp paired–end reads, resulting in a minimum of 20 GB of data per sample. To eliminate human host sequencing reads, raw reads were first screened against the human reference genome (GRCh38, August 2020) using Fastq Screen (v0.14.1) [24]. Reads with missing pairs, typically discarded in the host removal step, were corrected using BBMap (v38.84) [25]. Adapter sequences and low–quality reads were further filtered out using AfterQC (v0.9.7) with default parameters [26].

Taxonomic classification of microbial reads was performed using KrakenUniq (v0.5.7) [27], employing a custom database built from complete bacterial and archaeal genomes in RefSeq (January 2020). To increase classification accuracy, the krakenuniq–filter script was applied with a minimum unique k–mer threshold of 0.05. Taxonomic reports were generated using the krakenuniq–report. Resistome profiling was conducted using the AMRPlusPlus pipeline (v3.0.6) [28], which annotates ARGs based on the MEGARes database. Only ARGs with ≥80% gene coverage were retained for the analysis, and those requiring single nucleotide polymorphism (SNP) validation were excluded. ARG abundance was normalized using the following formula: [29].

$$\text{Abundance} = \sum_1^n \frac{N_{\mathrm{ARG-likesequence}} \times L_{\mathrm{reads}}/L_{\mathrm{ARGreferencesequence}}}{N_{\mathrm{16Ssequence}} \times L_{\mathrm{reads}}/L_{\mathrm{16Ssequence}}}$$

where $N_{\mathrm{ARG-likesequence}}$ and $N_{\mathrm{16Ssequence}}$ represent the number of reads mapped to ARGs and 16S rRNA genes, respectively; $\mathrm{L}_{\mathrm{reads}}$, $\mathrm{L}_{\mathrm{ARGreferencesequence}}$ and $\mathrm{L}_{\mathrm{16Ssequence}}$ are the lengths of sequencing reads, ARG reference sequences, and average 16S rRNA sequences, respectively. The number of 16S rRNA genes was quantified using Metaxa2 (v2.2) [30].

Diversity analysis and statistical test

To assess microbial and resistome diversity, count data from AMRPlusPlus and Kraken were normalized using Cumulative Sum Scaling (CSS) via the metagenomeSeq R package (v1.50.0) in R (v4.5.1) [31], to account for library size variation and compositional bias. For alpha diversity, normalized abundance values were used to compute Shannon and Simpson diversity indices, as well as evenness metrics, using the Phyloseq package (v1.53.2) [32]. Beta diversity was evaluated using Bray–Curtis dissimilarity matrices, followed by non–metric multidimensional scaling (NMDS) to visualize inter–sample variation. Differences between groups (e.g. Thai vs. Norwegian) were tested for statistical significance using permutational multivariate analysis of variance (PERMANOVA) as implemented in the vegan package (v2.7.1) [33]. All custom R scripts used for data processing, analysis, and figure generation are publicly available on GitHub: https://github.com/skittikun/oral_microbiome.git.

Community and resistance type clustering and ordination

To cluster genus level from Kraken and antimicrobial resistance gene (ARG) class relative abundances into community and resistance type, respectively, we implemented Dirichlet Multinomial Mixtures (DMM) [34] in R via DirichletMultinomial (v3.22) [35]. The optimal number of clusters was selected based on the Laplace, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC). The relative abundances from Kraken and ARG relative abundances were used to generate Bray–Curtis dissimilarity matrices in R via adonis in the vegan package (v2.7.1) [33]. Additionally, associations between taxonomy and resistome profiles were evaluated by calculating Spearman's rank correlation coefficients between the 15 most abundant genera and ARG classes, using the *Hmisc* package (v5.2–3) [36].

Data availability

All metagenomic sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1300960, and the individual accession numbers for each sample are provided in Supplementary Table S1.

Results

Sequencing output and data characteristics

A total of 50 Norwegian and 43 Thai saliva samples were subjected to metagenomic sequencing for comparative resistome and microbiome analysis. The modestly smaller Thai cohort resulted from the unavailability of MetaPolyzyme enzyme during part of the COVID–19 pandemic period, which precluded DNA extraction from an additional seven Thai samples. Although sequencing these samples without enzymatic pre–treatment was done, our prior study demonstrated that omission of MetaPolyzyme treatment leads to substantial shifts in both taxonomic and resistome profiles [23]. To ensure methodological consistency and maintain the validity of cross–cohort comparisons, these samples were excluded from downstream analyzes.

Table 1. Demographic and sequencing characteristics of study participants.

Metric	Thailand (*n* = 43)	Norway (*n* = 50)	Combined (*n* = 93)
DNA conc. (ng/µL)	18.3 ± 9.9 (4.4–40.6)	23.6 ± 13.1 (7.9–59.0)	21.2 ± 12.0 (4.4–59.0)
Raw reads (M)	143.1 ± 2.1 (140.3–145.5)	156.9 ± 8.8 (124.4–173.1)	150.5 ± 9.6 (124.4–173.1)
Non–human reads (M)	50.4 ± 21.2 (10.9–88.3)	50.0 ± 24.4 (9.5–100.2)	50.2 ± 22.9 (9.5–100.2)
Post–QC reads (M)	50.2 ± 21.1 (10.8–88.0)	49.7 ± 24.4 (9.4–99.8)	49.9 ± 22.8 (9.4–99.8)

Table 1 summarizes the sequencing characteristics of study participants. Sequencing yielded an average of 150.5 million reads per sample (range: 124.4–173.1 million). Following human DNA filtering, an average of 33.3% non–human reads per sample remained, which were further processed through quality control pipelines to produce a final average of 49.9 million high–quality paired–end reads per sample (range: 9.38–99.8 million). These data provided robust coverage for a comprehensive assessment of ARG and microbial community compositions in all included saliva samples. Detailed sequencing metrics for each sample are summarized in Supplementary Table S1.

Overview of the oral resistome

The composition and distribution of ARGs in the oral metagenomes of healthy individuals from Thailand and Norway are summarized in Figure 1. ARG relative abundances were calculated using normalized counts to account for differences in sequencing depth and gene length. At the individual sample level (Figure 1A), the resistome was predominantly composed of genes conferring resistance to macrolides–lincosamides–streptogramin (MLS), tetracyclines, and aminoglycosides, which together accounted for the largest proportion of ARGs in both populations. Thai samples appeared to exhibit

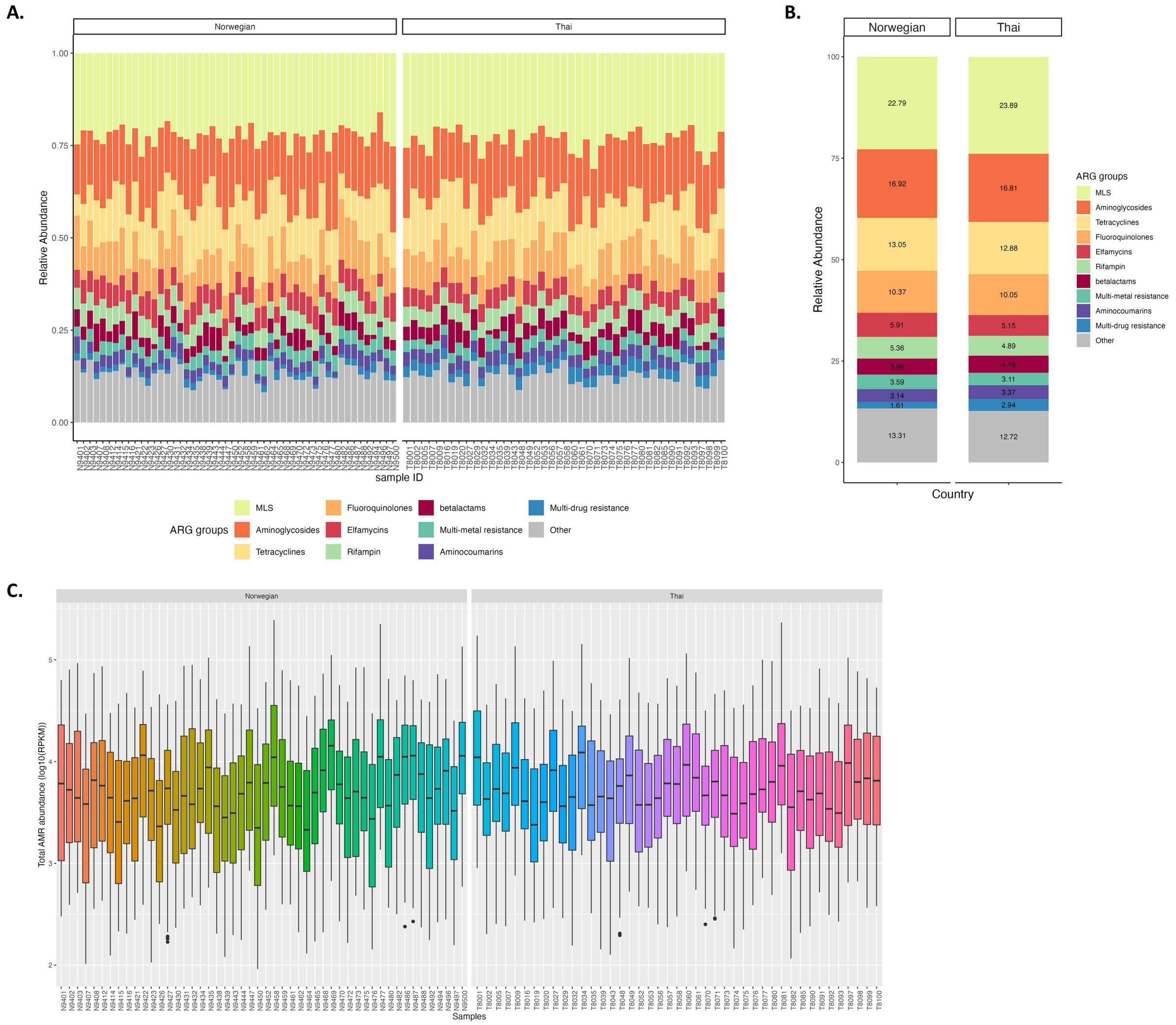

Figure 1. Composition and distribution of antimicrobial resistance genes (ARGs) in the oral microbiomes of Thai and Norwegian individuals. (A) Stacked bar plots displaying the relative abundances of ARG groups across individual samples. (B) Average relative abundances of ARG groups aggregated by country. (C) Boxplots of ARG abundances normalized by RPKM for samples from each country.

relatively higher contributions of MLS and tetracycline resistance genes, while Norwegian samples displayed consistently prominent proportions of aminoglycoside resistance genes.

When aggregated by country (Figure 1B), these trends were confirmed, with Thai samples showing slightly higher average relative abundances of MLS (23.89% vs. 22.79%) and tetracycline (16.81% vs. 16.02%) resistance genes compared to Norwegian samples, while aminoglycoside resistance genes were marginally more abundant in Norwegian samples (13.05% vs. 12.88%). Beta–lactam resistance genes and other groups, including multi–metal, rifampin, and drug and biocide resistance genes, comprised smaller fractions of the resistome in both populations. Analysis of total normalized ARG abundances per sample (Figure 1C), expressed as reads per million per kilobase (RPMK) to account for differences in sequencing depth and gene length, further revealed broadly comparable distributions and overlapping interquartile ranges. This indicates that the observed differences in resistome composition were not simply due to higher overall ARG loads in one population.

Differential abundance of resistance genes

To further characterize differences in the oral resistome profiles between the two populations, differential abundance analyzes were performed to identify specific ARG groups and individual genes driving the geographic patterns observed in Figure 1 using Norwegian samples as the reference group, such that positive $\log_2$ fold changes indicate enrichment in Thai samples. At the ARG group level (Figure 2A), Norwegian individuals were significantly enriched in aminoglycoside, sulfonamide, and quaternary ammonium compound (QAC) resistance genes, whereas Thai samples exhibited higher abundances of multi–biocide, nucleoside, and copper resistance gene groups.

Analysis at the individual ARG level (Figure 2B) revealed consistent patterns. Norwegian samples displayed significantly elevated abundances of multiple tetracycline resistance genes (*tetQ*, *tetO*, *tetW*, *tetA(46)*, *tetB(46)*, *tetT*), along with the macrolide resistance gene *msrD* and the QAC resistance gene *galE*. Conversely, Thai samples showed greater enrichment of aminoglycoside resistance genes such as *aph (2'')-Ia*, *aph(3')-III*, and *sat*, as well as macrolide genes including *ermB*, *ermF*, and *lsaC*. Together, these findings highlight distinct resistome signatures between Thai and Norwegian individuals, with broader differences at the ARG group level.

Resistome diversity and community structure

To investigate differences in resistome diversity between the two populations, we assessed alpha diversity using several indices derived from normalized ARG abundances (Figure 3A). Thai samples exhibited significantly greater ARG richness and evenness (both $p < 0.001$), indicating a broader repertoire of resistance genes and a more balanced resistome composition compared to Norwegian samples. These patterns were further supported by higher values of Shannon and inverse Simpson indices in Thai individuals ($p < 0.001$), confirming that the oral resistome in this population was not only richer but also more evenly distributed.

Beta diversity analysis based on Bray–Curtis dissimilarity revealed clear differences in resistome community structure between the two populations. NMDS ordination showed distinct clustering by countries (Figure 3B), with PERMANOVA confirming that population origin significantly explained variation in resistome profiles ($p = 0.001$, $R^2 = 0.149$). To visualize abundance patterns across samples, we generated a heatmap of ARG group abundances (Figure 3C), which revealed clustering patterns broadly separating Thai and Norwegian samples, consistent with differences observed in alpha and beta diversity. Thai samples tended to associate with a wider range of resistance groups, whereas Norwegian samples showed enrichment in particular ARG subsets. A similar pattern was observed in the top 40 gene–level heatmap (Supplementary Figure S1).

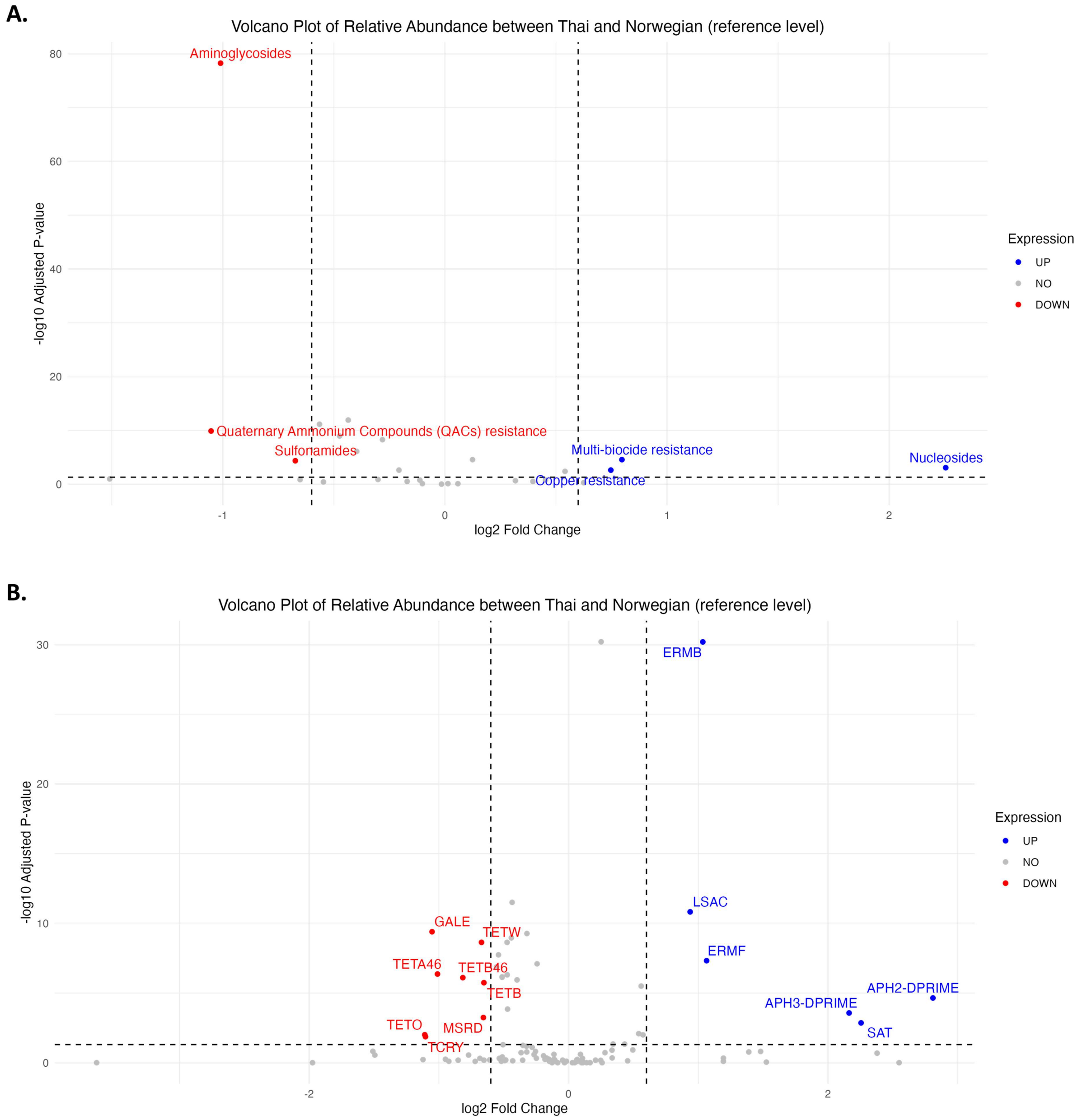

Figure 2. Differential abundance analyzes of ARGs between Thai and Norwegian oral samples. (A) Volcano plot illustrating log2 fold changes and adjusted *p*-values for individual ARGs, using Norwegian samples as the baseline. (B) Volcano plot of differential abundance at the ARG group level.

Oral microbiome composition and diversity

Analysis of the taxonomic composition revealed that the oral microbiome of both populations was predominantly comprised of genera such as *Streptococcus*, *Veillonella*, *Neisseria*, and *Prevotella* (Figure 4A). Although these core taxa were shared, their relative contributions varied by country. When averaged across samples, Norwegian samples exhibited higher proportions of *Prevotella*, *Streptococcus*, and *Veillonella*, whereas Thai samples showed relatively increased abundances of genera such as *Actinomyces* and *Haemophilus* (Figure 4B). Alpha diversity assessments based on normalized taxonomic profiles indicated that richness was significantly greater in Thai samples ($p < 0.001$), reflecting a higher number of observed taxa (Figure 4C). In contrast, evenness, Shannon, and inverse Simpson indices did not differ significantly between the two populations ($p > 0.05$), suggesting that while Thai samples

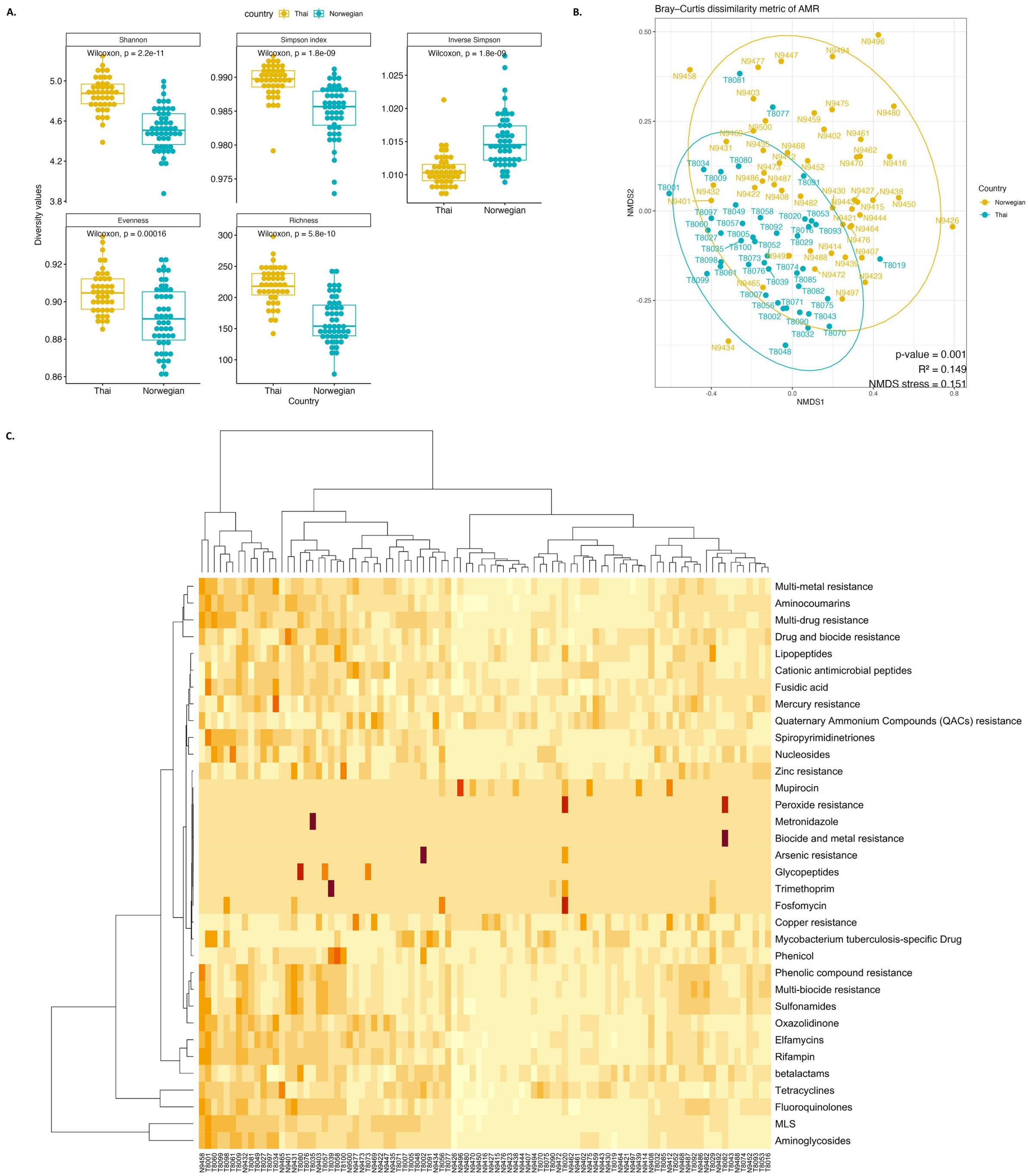

Figure 3. Diversity and community structure of the oral resistome. (A) Boxplots of alpha diversity metrics (richness, evenness, Shannon index, and inverse Simpson index) calculated from normalized ARG abundances. (B) NMDS ordination based on Bray–Curtis dissimilarities of ARG profiles. (C) Heatmap of ARG group–level abundances across samples. Warmer colors indicate higher relative abundance.

harbored a greater variety of taxa, the overall balance and dominance structures within these communities were comparable.

Beta diversity analysis using NMDS ordination of Bray–Curtis distances demonstrated distinct clustering by country (Figure 4C), with PERMANOVA confirming that population origin was a significant determinant of oral microbiome composition ($p = 0.001$, $R^2 = 0.099$). Differential abundance analysis at the genus level, using Norwegian samples as the reference group, further identified specific taxa associated with each population (Figure 4D). Notably, *Stenotrophomonas* and *Synechococcus* were enriched in

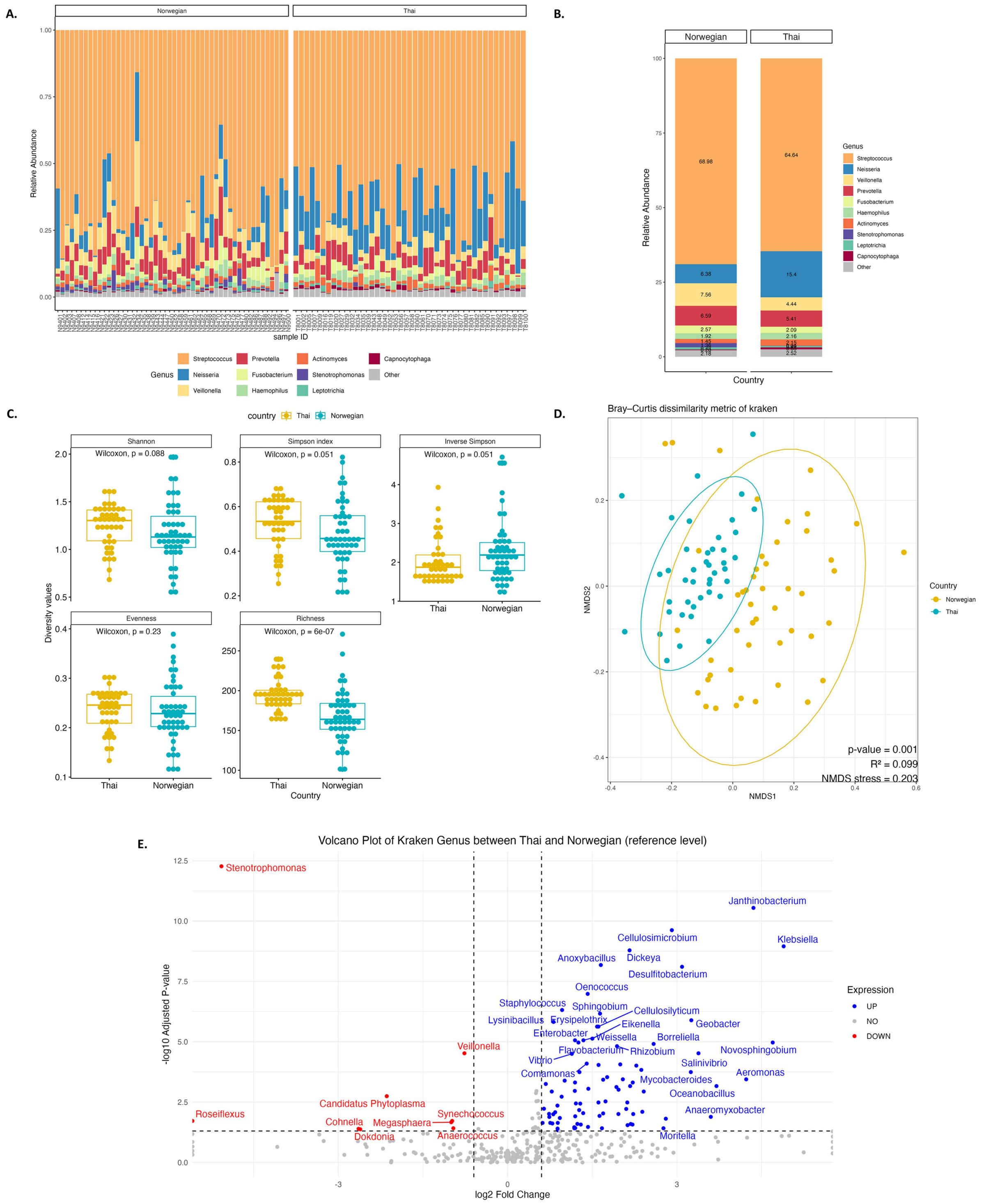

Figure 4. Taxonomic composition and diversity of the oral microbiome. (A) Stacked bar plots of the relative abundance of the top 10 most prevalent bacterial genera across individual samples. (B) Stacked bar plot of the cumulative relative abundance of top 10 most prevalent bacterial genera in two countries. (C) Boxplots of alpha diversity metrics (richness, Shannon index, and inverse Simpson index) derived from taxonomic profiles. (D) NMDS ordination of taxonomic community profiles based on Bray–Curtis dissimilarities. (E) Volcano plot of differentially abundant genera between Thai and Norwegian samples.

Norwegian samples, whereas higher abundances of genera such as *Janthinobacterium*, *Cellulosimicrobium*, and *Klebsiella* characterized Thai samples.

Clustering of community and resistome types

Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities revealed clear clustering of both taxonomic communities and resistome profiles. Full sample assignments for each community and resistome type are provided in Supplementary Tables S2 and S3, respectively. Two primary community types emerged (Figure 5A and Supplementary Table S2). Community type 1 was enriched in *Streptococcus*, *Prevotella*, and *Veillonella*, whereas community type 2 was also *Streptococcus*-dominated but had higher proportions of *Neisseria* and *Fusobacterium*. Norwegian samples (circles) were more often assigned to community type 1 (purple) and Thai samples (triangles) to type 2 (yellow), although considerable overlap was present (PERMANOVA $R^2 = 0.382$, $p = 0.001$). As summarized in Table 2, community type 1 consisted of 38 Norwegian and 14 Thai samples, while type 2 comprised 12 Norwegian and 29 Thai samples.

In contrast, resistome profiles exhibited a more pronounced separation by country (Figure 5B and Supplementary Table S3). Resistome type 1 (Lower–abundance/narrow–profile) and Resistome type 2 (Higher–abundance/broader–profile) differed in both the total number of ARG groups and the relative abundance of the dominant ARG groups (MLS, aminoglycoside, tetracycline, fluoroquinolone, and elfamycin). On average, Type 2 carried 21.4% more ARG groups and 22.5% higher relative abundances for these top five classes compared with Type 1. Although classification was based solely on resistome composition, a strong geographic trend emerged: type 1 included 45 Norwegian and only 3 Thai samples, whereas type 2 comprised 40 Thai and 5 Norwegian samples (Table 2; PERMANOVA $R^2 = 0.639$, $p = 0.001$). This pattern suggests that resistome composition may be more strongly structured by geographic or local selective pressures than overall microbial community composition.

Association between microbiome and resistome

To explore potential relationships between microbial taxa and ARGs, co–occurrence analysis was conducted using correlation matrices (Figure 5C and Supplementary Figure S2), which revealed that individual resistance gene groups co–occurred with distinct sets of bacterial genera, highlighting structured ecological interactions within the oral microbiome. Aminocoumarin resistance genes showed coordinated positive associations with *Aggregatibacter*, *Haemophilus*, *Gemella*, and *Neisseria* ($r \approx 0.31$–0.43), with the first three also strongly linked to fluoroquinolone resistance ($r \approx 0.49$–0.55). This cluster of genera consistently exhibited negative correlations with multi–metal, oxazolidinone, and aminoglycoside resistance determinants ($r \approx -0.28$ to -0.58), suggesting co–selection or niche exclusion processes shaping these resistome modules. Notably, *Neisseria* uniquely demonstrated additional positive correlations with multi–drug (0.44) and MLS resistance genes (0.35).

Beta–lactam resistance genes were predominantly associated with *Prevotella*, *Veillonella*, and *Schaalia* ($r \approx 0.33$–0.67), while *Stenotrophomonas* exhibited a distinct profile, marked by a strong negative correlation with multi–drug resistance (−0.67), moderate negative associations with multi–metal and oxazolidinone genes (both −0.31), and a positive correlation with elfamycin resistance (0.49), reflecting a specialized ecological niche potentially influenced by localized selective pressures. Additionally, *Lactobacillus* showed modest inverse associations with multi–metal and oxazolidinone resistance (both −0.30).

Together, these structured taxon–ARG co–occurrence patterns underscore the complex ecological and evolutionary processes, such as co–selection, horizontal gene transfer, and niche filtering, that may underlie the distinct resistome architectures observed between the Thai and Norwegian oral microbiomes.

Discussion

This study provides a direct metagenomic comparison of oral resistomes across two geographically and socioculturally distinct populations using standardized sampling, sequencing, and analytical approaches. To our knowledge, this is one of the first harmonized cross–country metagenomic datasets targeting the human oral resistome, enabling robust comparison between healthy individuals from Thailand and

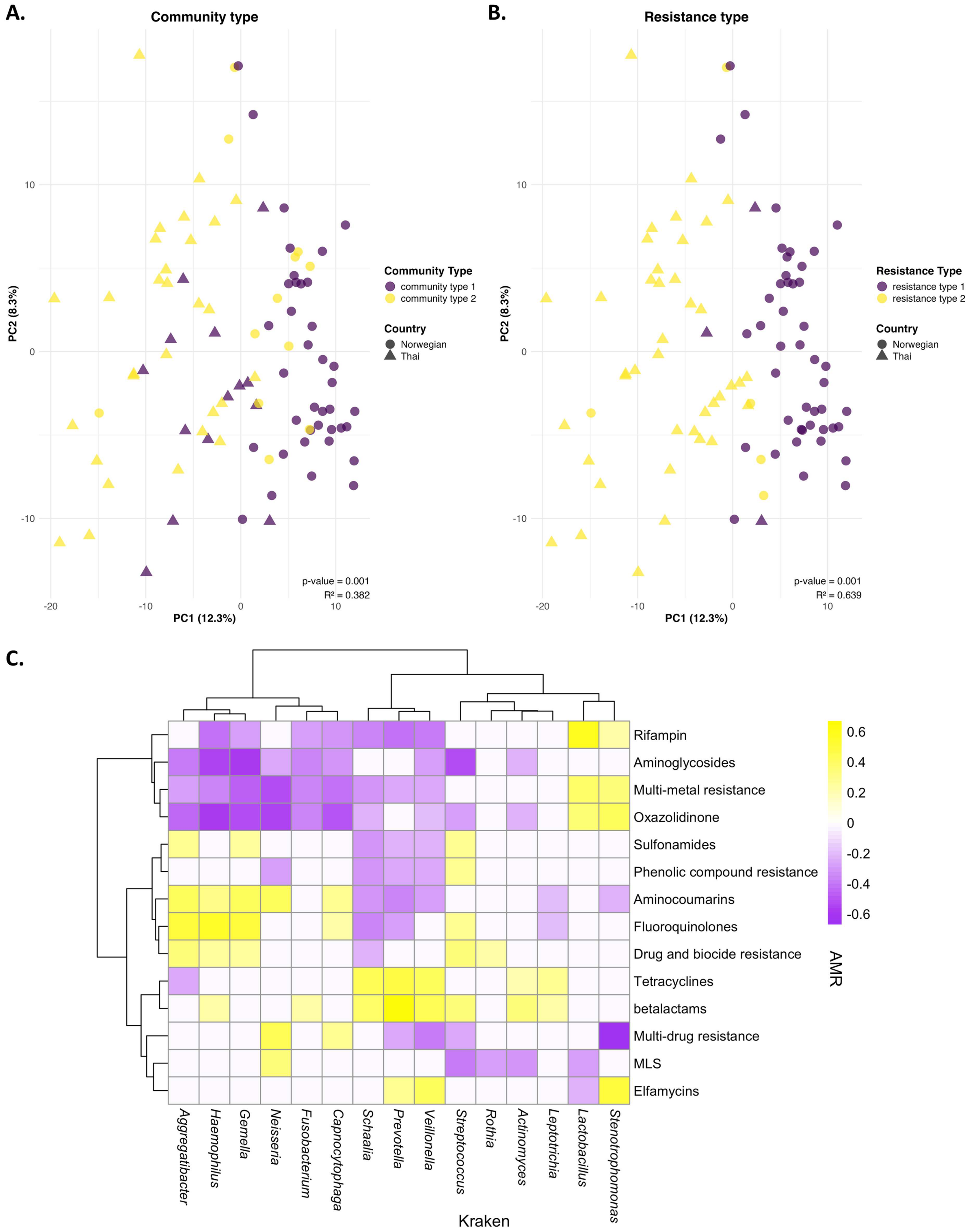

Figure 5. Integrative analysis of microbiome and resistome structuring and taxon–ARG associations. (A) PCoA of oral community composition (Bray–Curtis dissimilarity) showing two major community types: type 1 (*Streptococcus/Prevotella/Veillonella*-rich) and type 2 (*Streptococcus/Neisseria/Fusobacterium*-rich). (B) PCoA of resistome composition (Bray–Curtis dissimilarity) showing two major resistome types: type 1 (Lower–abundance/narrow–profile) and type 2 (Higher–abundance/broader–profile). Shapes indicate country of origin; colors indicate assigned type. (C) Heatmap of correlation coefficients depicting co–occurrence relationships between ARG groups and bacterial genera.

Norway. We observed clear biogeographic differences in resistome structure and diversity between healthy individuals from Thailand and Norway. Thai samples exhibited significantly higher resistome richness,

Table 2. Distribution of microbiome community types and resistance types among Norwegian and Thai samples.

Profiles	Type & description	Norwegian	Thai	Total
Community	Type 1 (*Streptococcus*/*Prevotella*/*Veillonella*-rich)	38	14	52
	Type 2 (*Streptococcus*/*Neisseria*/*Fusobacterium*-rich)	12	29	41
Resistance	Type 1 (Lower–abundance/narrow–profile)	45	3	48
	Type 2 (Higher–abundance/broader–profile)	5	40	45

evenness, and overall diversity, driven primarily by elevated abundances of MLS, tetracycline, and multi–biocide resistance genes. In contrast, Norwegian samples showed stronger enrichment of aminoglycoside, sulfonamide, and quaternary ammonium compound (QAC) resistance genes. These findings align with previous work suggesting that geographic, behavioral, and healthcare–related factors strongly influence human resistome composition, even in the absence of overt disease or recent antibiotic use [37–39]. Notably, the stronger geographic structuring observed for resistomes (PERMANOVA $R^2 = 0.639$) compared with microbiomes ($R^2 = 0.382$) suggests that factors beyond taxonomic composition, such as local selective pressures, antimicrobial usage patterns, and horizontal gene transfer, may play a more dominant role in shaping ARG distribution across populations. Recent oral metagenomic studies have shown that the oral cavity harbors a structured resistome across both healthy and diseased states, with many ARGs predicted to be located on mobile genetic elements such as plasmids [10].

Comparable dominant ARG classes have been reported in other recent oral resistome studies. Anderson et al. [10] identified tetracycline, MLS, and β-lactam resistance as the most prevalent groups in oral biofilms across health and disease states, with common determinants such as *mef(A)*, *msr(D)*, *erm(F)*, *cfxA*, and *tet(Q)* [10]. Similarly, Bartsch et al. [16] observed enrichment of tetracycline efflux genes (*tetA*/*tetB*) and MLS–associated determinants following chlorhexidine exposure in healthy adults [16]. Our study similarly observed these core resistance signatures, particularly MLS and tetracycline genes, while extending the comparison across distinct geographic and sociocultural contexts. Whereas the previous study linked resistome types primarily to oral health status or antiseptic exposure, our resistome types reflect geographic structuring, suggesting that regional selective pressures (e.g. antibiotic usage, hygiene products, or environmental exposure) can drive ARG distribution independently of disease state. Together, these studies reinforce the notion of a globally conserved but contextually modulated oral resistome [10,16].

While taxonomic differences were also observed between the two cohorts, they did not fully explain the divergence in resistome profiles. Principal coordinate analysis demonstrated that resistome composition was more strongly structured by country rather than by overall microbial community composition, suggesting that selective pressures, gene mobility, or environmental exposures may shape the resistome independently of taxonomic shifts. Specifically, the country explained a higher proportion of variance in resistome profiles than in microbial taxa, indicating that ARG distribution is not solely a function of which microbes are present. Co–occurrence analysis supported this interpretation, revealing structured associations between certain ARG groups and a wide range of bacterial genera, rather than tight one–to–one relationships. Such patterns are consistent with horizontal gene transfer, ecological filtering, or co–selection mechanisms acting across diverse microbial backgrounds [40,41].

Several environmental, behavioral, and healthcare–related factors likely contribute to the resistome divergence observed between Thai and Norwegian participants. Non–antibiotic antimicrobials such as chlorhexidine have also been shown to alter oral microbial ecology and ARG composition [16], supporting our interpretation that local hygiene practices and antimicrobial exposures can modulate resistome architecture even in healthy individuals. Differences in national antibiotic stewardship policies are probably one of the key factors. Thailand has historically reported higher antibiotic consumption and over–the–counter access, whereas Norway maintains a stringent prescription–only use and strong public health campaigns promoting antimicrobial stewardship. In fact, national surveillance data indicated that antibiotic use in Norway has been declining steadily, with sales statistics from primary care, hospitals and nursing homes showing a 22% reduction between 2012 and 2019 [42]. The Norwegian National One

Health Strategy now aims to further cut human antibiotic use from about 450 to 250 prescriptions per 1,000 inhabitants (44%) by 2030, alongside curbing broad–spectrum use and strengthening AMR surveillance [43]. This regulatory contrast has been associated with differing prevalence of resistance genes at the population level, as demonstrated by large–scale metagenomic studies of national wastewater, which show higher ARG abundance in countries with more liberal antibiotic access and use [44]. The timing of this comparative analysis is particularly relevant, as both Thailand and Norway are currently revising national AMR action plans under the WHO's Global Action Plan framework. Cross–country oral resistome data generated with standardized methods can provide baseline references to monitor the impact of these policy changes and detect emerging resistance trends before they appear in clinical or environmental reservoirs.

Diet and hygiene practices may also play a role; high sugar intake, fermented foods, and the use of antimicrobial oral hygiene products can shape both the oral microbiome and resistome. Moreover, sociocultural behaviors such as tobacco use, chewing habits, or frequency of dental visits could influence microbial composition and exposure to antimicrobials. While our study did not directly capture these variables, their potential influence undoubtedly underscores the value of integrating epidemiological and behavioral data in future studies. From a dental public health perspective, these insights may help inform antibiotic stewardship strategies in oral healthcare, where targeted measures, such as optimizing prophylactic antibiotic use in dental procedures and promoting evidence–based antimicrobial oral hygiene practices, could contribute to mitigating the proliferation of ARGs in the oral cavity. As this was a cross–sectional analysis, our findings reflect associations rather than causation, and longitudinal or interventional studies would be needed to directly assess the drivers of the observed resistome differences.

Our findings are consistent with and extend previous studies demonstrating that even in healthy populations, the resistome is not randomly distributed but is shaped by ecological and sociocultural factors. For example, a recent global–scale analysis of oral resistome studies by Sukumar et al. [6] highlighted regional differences in ARG prevalence across populations but noted that methodological heterogeneity limited direct comparison [6]. Our study addresses this by applying harmonized protocols across two countries, enabling more robust cross–population comparisons. Furthermore, by linking resistome and taxonomic patterns through correlation–based ecological mapping, we provide a deeper mechanistic understanding of ARG distribution within the oral niche.

Despite these insights, several limitations should be acknowledged. The use of short–read metagenomics restricts our ability to resolve the genetic context of ARGs, such as their association with plasmids, integrons, or specific bacterial host strains, which limits inferences about horizontal gene transfer potential. Although normalization methods (e.g. RPKM) were applied, subtle biases from differential DNA extraction efficiency or unmeasured exposures may persist. Moreover, sequencing–based detection of ARGs does not necessarily imply phenotypic resistance, as gene expression and activity depend on microbial host and environmental conditions.

The sample size, while modest, was sufficient to detect consistent microbiome and resistome clustering patterns across cohorts collected under harmonized protocols. As this study was cross–sectional, it emphasized comparative population–level trends rather than individual variation, and could not assess the temporal stability of the oral resistome. Detailed clinical and environmental metadata, such as diet, oral hygiene behavior, and previous antibiotic use, were not captured and may contribute to the observed inter–population differences. Additionally, as oral health status was based on self–report rather than formal dental examination, subclinical or undiagnosed oral conditions (e.g. early periodontal disease) cannot be excluded. This limitation highlights the importance of integrating standardized dental assessments in future oral resistome studies. Future research incorporating strain–resolved or long–read metagenomics, longitudinal sampling, phenotypic resistance testing and functional validation will help clarify the functional and ecological factors shaping oral resistome diversity.

The broader implications of our findings are noteworthy. Although the oral cavity as a distinct microbial habitat is often overlooked in AMR surveillance, it may serve as both a reservoir and a transmission point for antimicrobial resistance genes. Its frequent exposure to environmental and pharmaceutical agents, as well as its role as a gateway to gastrointestinal and systemic circulation, positions the oral microbiome as a critical interface in the dissemination of AMR within and beyond the host. Understanding how resistomes are shaped by local ecology, antimicrobial practices, and lifestyle factors will be essential for developing context–appropriate AMR mitigation strategies.

Conclusions

This study demonstrates the value of applying ecological frameworks to resistome research. The distinct resistome architectures observed between Thai and Norwegian populations highlight the need for region–specific surveillance and emphasize the influence of environmental, behavioral, and policy–related factors on antimicrobial resistance dynamics. By integrating taxonomic, functional, and ecological analyzes, our findings provide a foundation for understanding how resistomes are structured within the oral microbiome. Given the oral cavity's role as a reservoir for diverse and potentially mobile ARGs, these results support its inclusion in future antimicrobial resistance surveillance and mitigation strategies, with oral resistome profiling incorporated into One Health frameworks to enable earlier detection of emerging resistance trends and their potential links to human, animal, and environmental reservoirs.

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Author contributions

S.T. and M.A. were involved in the design of experiments and conceptualizing. S.T., K.S., and W.L. were involved in data analysis, visualization, and interpretation of the results. S.T., J.W., E.W. and N.C. were involved in clinical sample collection and processing. S.T. drafted the manuscript, and all authors contributed towards the editing of the manuscript as well as the approval of the final manuscript.

Disclosure statement

The authors declare that there are no conflicts of interest.

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Data availability statement

All metagenomic sequencing data are deposited in NCBI SRA (BioProject PRJNA1300960), with accession numbers in Supplementary Table S1.

Ethical approval

The collection of saliva samples was approved by the Regional Committees for Medical and Health Research Ethics (Project number 2018/1373/REK nord) and the Ethical Review Sub–Committee Board of Human Research Involving Science, Thammasat University, Thailand (COA No. 163/2562). All samples were anonymized, and written consent was obtained before sample collection.

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