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Ecological relevance of oral Staphylococcus carriage in healthy adults

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Abstract

Aims

The oral cavity is increasingly recognized as a reservoir of Staphylococcus species, yet knowledge gaps remain regarding species diversity, antimicrobial resistance (AMR), overlap with nasal carriage, and colonization persistence. Therefore, this study characterized oral and nasal Staphylococcus colonization in healthy young adults and explored the oral cavity as a potential niche for antimicrobial resistance (AMR) surveillance.

Materials and methods

Staphylococcus spp. from oral and nasal samples of 101 dentistry students were isolated and identified by MALDI-TOF MS. Antimicrobial susceptibility was assessed using EUCAST/CLSI guidelines. Longitudinal oral samples from a subgroup were analysed by Fourier transform infrared spectroscopy (FTIR-ATR) to explore strain persistence and turnover.

Results

*Staphylococcus c*olonization was highly prevalent (oral: 91%; nasal: 95%), with 86% concurrent oro-nasal colonization. *Staphylococcus epidermidis* predominated in both niches. Resistance to ≥ 1 antimicrobial was detected in 80% of coagulase-negative staphylococci (CoNS) and 73% of oral *Staphylococcus aureus* carriers. Multidrug-resistant phenotypes were found in 10% of CoNS carriers. Although multidrug-resistant and methicillin-resistant *S. aureus* was not detected, oral *S. aureus* frequently showed resistance to commonly prescribed dental antibiotics (amoxicillin, erythromycin, and clindamycin). Resistance frequencies were comparable between niches. Species-specific colonization dynamics suggested temporal turnover among *Staphylococcus* warneri and persistence-like clustering for *S. aureus* in some participants.

Conclusions

The oral cavity harbours diverse, frequently antimicrobial-resistant *Staphylococcus* populations at rates comparable to the nasal cavity. These findings support the oral cavity as a complementary ecological niche for *Staphylococcus* carriage, highlighting its relevance for future AMR surveillance strategies in dentistry and human health.

1. Introduction

The oral cavity harbours a complex microbiome shaped by host factors, environmental conditions, and microbial interactions [1–3]. Composed of bacteria, microeukaryotes, archaea, and viruses, this ecosystem contributes to both health and disease. The anatomical diversity of the oral cavity supports multispecies biofilms across distinct microhabitats, enabling the coexistence of resident and transient microorganisms [1–3]. Within this environment, *Staphylococcus* spp. are increasingly recognised as members of the oral microbiota [2, 4–7], although their ecological role remains incompletely understood [4].

The genus *Staphylococcus* includes approximately 70 species, many of which commonly colonize the skin and anterior nares and may act as important pathogens [6, 8–10]. Although the anterior nares are considered the main reservoir for *Staphylococcus aureus* [8, 11], increasing evidence indicates that the oral cavity may also contribute to staphylococcal

carriage [6, 12–14]. Multiple *Staphylococcus* species have been detected in oral habitats, including healthy individuals [4–7], yet it remains unclear whether they represent transient colonizers continuously seeded from extra-oral reservoirs or whether are capable of long-term persistence.

This distinction has important implications for microbial ecology, host adaptation, transmission, and antimicrobial resistance maintenance [4, 9, 13].

Clarifying these colonization dynamics is particularly relevant given the opportunistic potential of both *S. aureus* and coagulase-negative staphylococci (CoNS) [15, 16].

From a clinical and public health perspective, the relevance of oral *Staphylococcus* spp. carriage is further amplified by antimicrobial resistance (AMR) [17–19]. Oral biofilms and repeated exposure to antibiotics may favour the maintenance and horizontal transfer of resistance genes within the oral ecosystem [12, 20–22]. Both *S. aureus* and CoNS have been implicated in the carriage of resistance genes, including multidrug resistance profiles and mobile genetic elements, highlighting the oral cavity as a potential reservoir for AMR dissemination [6, 22, 23]. In particular, methicillin-resistant *S. aureus* (MRSA) remain a major global health concern and was recognised by the World Health Organization as a priority pathogen in 2019 [24]. Beyond MRSA, phenotypically resistant CoNS may also be relevant because they can persist as commensals while contributing to the broader ecology of resistance [16, 22, 23].

Although previous studies have documented *Staphylococcus* spp. in the oral cavity, important gaps remain regarding the species-level diversity, overlap with nasal carriage, phenotypic AMR among CoNS, and temporal colonization stability [4, 6, 7, 13]. Accordingly, this study characterized oral *Staphylococcus* colonization in healthy young adults, assessing species diversity, antimicrobial susceptibility, and longitudinal colonization dynamics to distinguish transient from persistent oral carriage. By integrating oral and nasal sampling with resistance profilling and exploratory longitudinal strain typing, we evaluated the oral cavity as a complementary niche staphylococcal carriage and AMR surveillance.

2. Materials and Methods

A total of 101 healthy dental students from the Faculty of Dental Medicine of the University of Porto (FMDUP, Portugal) were recruited. The study was approved by the FMDUP Ethics Committee (Project No. 19/2025), and all participants provided written informed consent. Demographic, oral health, and recent medical history data were collected through a questionnaire. Individuals reporting antibiotic use or infection within the previous 6 months were excluded. Oral and nasal swabs were collected from all participants (n = 101). To explore colonization dynamics, oral samples were longitudinally collected from a subgroup of participants (n = 31) at up to four time points. Definitions of study variables, outcomes, and microbiological endpoints are provided in the Supplementary material.

Staphylococcal isolates were recovered using selective culture and identified by MALDI-TOF MS. Antimicrobial susceptibility was evaluated by the disk diffusion method according to EUCAST or CLSI recommendations, and multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. Longitudinal strain relatedness was assessed by FTIR-ATR (IR Biotyper®, Bruker) in participants carrying the same species at multiple time points. Statistical analyses were performed using SPSS v29.0. Group comparisons were conducted using appropriate parametric or non-parametric tests according to data distribution, and statistical significance was set at $P < 0.05$. Detailed methodologies are provided in the Supplementary Materials. This study was conducted and reported according to STROBE guidelines.

3. Results

The clinical and demographic information of the participants are presented in Supplementary Table S1. The studied population consisted predominantly of young adult females with generally good oral health, reflected by a low DMFT index and infrequent gingivitis or fixed prostheses.

A total of 364 *Staphylococcus* isolates were identified, distributed between the nasal (n = 203) and oral (n = 161) cavities (Supplementary Table S2). Colonization rates were high in both anatomical niches, affecting 95.1% of nasal cavities and 91.1% of oral cavities (Table 1). Ecological overlap was substantial, with 86.1% of individuals presenting simultaneous (oro-nasal) colonization. Among co-colonized individuals, *S. epidermidis* (46.5%) and *S. aureus* (9.9%) were the most prevalent colonizing species. *S. epidermidis* was the predominant species in both the oral (64.4%) and nasal (67.3%) cavities. In the oral cavity, the next most prevalent species were *S. aureus* (21.8%) and *S. warneri* (17.8%), whereas in the nasal cavity *S. aureus* and *S. warneri* (both 23.8%) were followed by *S. capitis* (19.8%).

Table 1
Oral and nasal *Staphylococcus* colonization rates among study participants.

***Staphylococcus* species**	**Oral & Nasal**		**Oral**		**Nasal**		***p***
	n	**%**	**n**	**%**	**n**	**%**	
Staphylococcus colonization	87	86.1%	92	91.1%	96	95.1%	*0.424*
Staphylococcus aureus	10	9.9%	22	21.8%	24	23.8%	*0.845*
Staphylococcus capitis	2	2.0%	7	6.9%	20	19.8%	*0.011**
Staphylococcus cohnii	1	1.0%	1	1.0%	1	1.0%	*> 0.999*
Staphylococcus carnosus group	0	0.0%	1	1.0%	0	0.0%	*> 0.999*
Staphylococcus epidermidis	47	46.5%	65	64.4%	68	67.3%	*0,749*
Staphylococcus haemolyticus	0	0.0%	3	3.0%	3	3.0%	*> 0.999*
Staphylococcus hominis	0	0.0%	2	2.0%	4	4.0%	*0.687*
Staphylococcus lugdunensis	0	0.0%	2	2.0%	3	3.0%	*> 0.999*
Staphylococcus pasteuri	2	2.0%	10	9.9%	4	4.0%	*0.109*
Staphylococcus pettenkoferi	0	0.0%	2	2.0%	0	0.0%	*0.500*
Staphylococcus saprophyticus	3	3.0%	6	5.9%	15	14.9%	*0.035**
Staphylococcus warneri	5	5.0%	18	17.8%	24	23.8%	*0.377*

*p < 0.05, statistical significance differences in colonization frequencies between paired oral and nasal cavity samples, determined using the exact McNemar's test. n, number of participants colonized; %, percentage of participants colonized.

The distribution of the different *Staphylococcus* species identified in the oral and nasal cavities is represented in Fig. 1, highlighting both overlapping and niche-specific species. Colonization frequencies were similar between niches for overall *Staphylococcus* carriage and most species. However, *Staphylococcus capitis* and *Staphylococcus saprophyticus* were significantly more frequent in nasal samples than in oral samples. *Staphylococcus carnosus* group and *Staphylococcus pettenkoferi* were recovered only from oral samples (Table 1).

No significant differences in oral *Staphylococcus* colonization frequencies were observed between caries-free participants (DMFT = 0) and those with previous caries experience (DMFT > 0) for any of the identified species (all p > 0.05) (Supplementary Table S3). Also, DMFT scores were compared between participants with and without oral *S. aureus* colonization, and no statistically significant difference was found (2.67 ± 2.38 vs. 2.58 ± 2.69; Mann-Whitney, *p = 0.693*). Due to the low prevalence of gingivitis and the low number of participants using dental prostheses, no statistical analysis was performed for these variables.

Antimicrobial susceptibility testing revealed frequent resistance among oral and nasal *Staphylococcus* isolates (Tables 2 and 3). Among participants carrying coagulase-negative staphylococci (CoNS), resistance to at least one antimicrobial agent was highly prevalent, ranging from 43% to 100% depending on the species. The MDR phenotype was observed in isolates from S. cohnii, S. epidermidis, S. haemolyticus, and S. hominis species.

Table 2
Frequency of antimicrobial resistance and multidrug resistance among oral and nasal *Staphylococcus* carriers.

***Staphylococcus* species**	**Oral**			**Nasal**			***p* (R)**	***p* (MDR)**
	n	**R (%)**	**MDR (%)**	**n**	**R (%)**	**MDR (%)**		
Staphylococcus aureus	22	73%	0%	24	79%	4%	*0.725*	*> 0.999*
Staphylococcus capitis	7	43%	0%	20	50%	0%	*> 0.999*	-
Staphylococcus cohnii	1	100%	100%	1	100%	0%	-	*> 0.999*
Staphylococcus carnosus group	1	100%	0%	0	0%	0%	-	-
Staphylococcus epidermidis	65	82%	14%	68	79%	10%	*0.811*	*0.596*
Staphylococcus haemolyticus	3	100%	33%	3	100%	33%	-	*> 0.999*
Staphylococcus hominis	2	100%	50%	4	100%	75%	-	*> 0.999*
Staphylococcus lugdunensis	2	50%	0%	3	67%	0%	*> 0.999*	-
Staphylococcus pasteuri	10	70%	0%	4	75%	0%	*> 0.999*	-
Staphylococcus pettenkoferi	2	100%	0%	0	-	-	-	-
Staphylococcus saprophyticus	6	83%	0%	15	87%	0%	*> 0.999*	-
Staphylococcus warneri	18	83%	0%	24	76%	4%	*0.491*	*> 0.999*

P-values were calculated using Fisher's exact test. R, resistant to one antimicrobial agent; MDR, Multidrug resistance - resistant to three or more antibiotic classes.

Table 3

Percentage of antimicrobial resistance among *Staphylococcus* isolates recovered from oral and nasal cavities.

***Staphylococcus* species**			**% Antibiotic Resistance**									
	Niche	**n**	**AMO**	**CXI**	**CIP**	**CHL**	**GEN**	**TET**	**TRS**	**ERY**	**CLI**	**QUD**
S. aureus	Oral	24	46%	0%	0%	0%	58%	4%	0%	29%	29%	0%
	Nasal	27	41%	0%	0%	0%	74%	4%	0%	33%	33%	0%
CoN	Oral	137	28%	3%	1%	0%	50%	9%	15%	41%	15%	-
	Nasal	176	28%	2%	0%	1%	44%	11%	11%	44%	15%	-
S. capitis	Oral	7	0%	0%	0%	0%	0%	0%	0%	43%	14%	-
	Nasal	22	9%	0%	0%	0%	23%	5%	5%	36%	18%	-
S. cohnii	Oral	1	0%	0%	0%	0%	100%	100%	100%	100%	100%	-
	Nasal	1	0%	0%	0%	0%	0%	0%	0%	100%	0%	-
S. carnosus group	Oral	1	0%	0%	0%	0%	100%	0%	0%	0%	0%	-
	Nasal	0	-	-	-	-	-	-	-	-	-	-
S. epidermidis	Oral	80	31%	3%	1%	0%	51%	13%	23%	44%	18%	-
	Nasal	91	32%	2%	0%	0%	49%	11%	13%	44%	14%	-
S. haemolyticus	Oral	3	67%	33%	0%	0%	0%	0%	33%	100%	33%	-
	Nasal	3	0%	0%	0%	0%	33%	0%	67%	67%	0%	-
S. hominis	Oral	2	0%	0%	0%	0%	50%	50%	50%	50%	50%	-
	Nasal	4	75%	50%	0%	0%	25%	50%	75%	75%	75%	-
S. lugdunensis	Oral	2	0%	0%	0%	0%	0%	0%	0%	50%	50%	-
	Nasal	4	50%	0%	0%	0%	25%	25%	0%	25%	25%	-
S. pasteuri	Oral	11	36%	0%	0%	0%	55%	0%	0%	36%	0%	-
	Nasal	4	50%	0%	0%	0%	75%	0%	25%	25%	0%	-
S. pettenkoferi	Oral	2	0%	50%	0%	0%	50%	0%	0%	0%	0%	-
	Nasal	0	-	-	-	-	-	-	-	-	-	-
S. saprophyticus	Oral	6	0%	0%	0%	0%	83%	0%	0%	0%	0%	-
	Nasal	17	12%	0%	0%	0%	76%	12%	0%	29%	18%	-
S. warneri	Oral	22	36%	0%	0%	0%	55%	5%	0%	36%	9%	-
	Nasal	30	33%	0%	0%	3%	27%	10%	3%	53%	10%	-

Results for Quinupristin-Dalfopristin (QUD) are presented exclusively for *S. aureus*, as current antimicrobial susceptibility testing guidelines (EUCAST/CLSI) only recommend testing and clinical breakpoints for this specific species. n, number of isolates. AMO, Amoxicillin; CXI, Cefoxitin; CIP, Ciprofloxacin; CHL, Chloramphenicol; GEN, Gentamicin; TET, Tetracycline; TRS, Trimethoprim-sulfamethoxazole; ERY, Erythromycin; CLI, Clindamycin; QUD, Quinupristin-dalfopris.

For *S. aureus*, resistance to at least one antimicrobial agent was observed in 73% oral carriers and 79% of nasal carriers, while MDR was detected only among nasal carriers (4%). No cefoxitin-resistant *S. aureus* isolates were detected, indicating the absence of MRSA among the analysed participants (Table 3).

Species-specific antimicrobial resistance profiles are summarized in Table 3. Overall, resistance was most commonly observed against gentamicin, amoxicillin, erythromycin, and clindamycin. Participants carrying S. *aureus* exhibited significantly higher frequencies of gentamicin- and clindamycin-resistant isolates than those carrying CoNS in nasal cavity (GEN: 74% vs. 44%, p = 0.004; CLI: 33% vs. 15%, p = 0.027, Fisher test). Conversely, resistant to trimethoprim-sulfamethoxazole in the oral cavity was significantly more frequently observed among CoNS cariers (TRS: 0% vs. 15%, p = 0.045, Fisher test).

No significant differences in resistance (R) or MDR frequencies were observed between oral and nasal isolates for any identified species (Table 2), indicating that the oral cavity harboured resistant *Staphylococcus* populations at frequencies comparable to those observed in the nasal cavity.

The temporal dynamics of *Staphylococcus warneri* and *S. aureus* strains were assessed by analysing FTIR-ATR spectra of isolates recovered from each participant at up to four sampling time points (T0, T1, T2, and T3). For both species, hierarchical clustering was performed using Euclidean distance and the average linkage algorithm, resulting in dendrograms with high cophenetic correlation coefficients of 0.919 for *S. warneri* and 0.862 for *S. aureus*, indicating a good fit between the dendrogram and the original distance matrix (*S. warneri* - Supplementary Figures S1&S2; *S. aureus* - Supplementary Figures S3&S4).

For *S. warneri*, the clustering cut-off value (0.160) was selected based on intra-sample similarity patterns, as isolates recovered from the same participant at the same time point consistently grouped below this threshold, supporting its use to define highly similar strain profiles within the dataset (Supplementary Figures S1&S2- isolates 50 and 79 from Participant 1 at T1). Using this threshold, *S. warneri* isolates displayed marked heterogeneity across time points, suggesting dynamic colonization patterns (Supplementary Figure S1&S2). This pattern suggests temporal turnover rather than stable persistence. This dispersion was supported by Principal Component Analysis (PCA), where six Principal Components (PCs) were sufficient to explain 95.2% of the variance, reflecting structured spectral variability among the isolates (Fig. 2).

In contrast, *S. aureus* displayed a more heterogeneous colonisation patterns across individuals. Using the same cut-off (0.160) for consistency, different temporal patterns were observed. In some participants (e.g., P3, P22, P26), isolates from two or more time-points clustered together, suggesting the persistence of similar strain profiles over time (Supplementary Figures S3&S4). In other participants (e.g., P29 and P4), isolates were distributed across different clusters, suggesting strain profile turnover between sampling points. These findings suggest that oral *S. aureus* colonization may be persistent in some individuals but transient or unstable in others. This variability *of S. aureus* was also reflected in the multivariate analysis, where 11 principal components (PCs) were required to explain for 95.2% of the variance, suggesting a complex distribution of spectral profiles (Fig. 2).

Overall, the FTIR-ATR exploratory analysis suggested species-specific colonization dynamics, with greater turnover among *S. warneri* isolates and persistence-like clustering of *S. aureus* isolates in a subset of participants.

4. Discussion

This study aimed to characterize oral *Staphylococcus* colonization in healthy young adults, including species diversity, antimicrobial resistance profiles, and longitudinal colonization dynamics. The main findings were that oral *Staphylococcus* carriage was highly prevalent, exhibited substantial overlap with nasal colonization, harboured

antimicrobial-resistant populations at frequencies comparable to the nasal cavity, and displayed species-specific temporal dynamics. Together, these findings support the oral cavity as a relevant ecological niche for *Staphylococcus* carriage and a potential target for antimicrobial resistance surveillance.

The results of this study highlight the relevance of the oral cavity as a frequently colonized ecological niche for *Staphylococcus* species, challenging the paradigm that limits it to a transit site resulting from aspiration or swallowing. The high oral colonization rate (91%), the strong oro-nasal overlap (86%), and recovery of more than 150 oral isolates across multiple species suggest that the oral cavity represents a viable reservoir for this genus. These findings are consistent with previous studies reporting oral carriage of *Staphylococcus* spp. in healthy individuals and strengthen emerging evidence that the oral cavity constitutes an additional reservoir for *S. aureus* and antimicrobial-resistant staphylococci [4, 6, 12, 13]. Interestingly, isolates belonging to the *S. carnosus* group and *S. pettenkoferi* were recovered exclusively from oral samples. However, given the limited number of isolates for these species, these findings should be interpreted cautiously. Overall, these observations support the growing evidence that the oral cavity may contribute to the ecological maintenance and dissemination of *Staphylococcus* species in healthy individuals.

The fact that colonization by these species is independent of the DMFT index suggests that oral staphylococcal carriage is not directly associated with dental caries. Although the relationship between *Staphylococcus* and dental caries remains unclear, this genus has recently been associated with adolescents without dental caries [25]. Together, these findings suggest that oral staphylococcal carriage may reflect ecological balance or exposure-related colonization rather than a direct marker of dental pathology.

Despite the mostly commensal nature of several species detected in this study, the observed antimicrobial susceptibility profiles are relevant from a public health perspective. The oral cavity harboured phenotypically resistant *Staphylococcus* populations at frequencies comparable to those observed in the nasal cavity, particularly among CoNS. The high frequency of resistance to at least one antimicrobial agent among *S. epidermidis* carriers, together with the detection of MDR phenotypes, highlights the importance of CoNS as reservoirs of phenotypic antimicrobial resistance in healthy individuals. This is particularly relevant because CoNS are often interpreted as low-virulence commensals, yet they may contribute to the maintenance and potential dissemination of resistance traits within human-associated microbial communities [16, 22, 23].

The absence of cefoxitin-resistant *S. aureus* indicates that MRSA was not detected in this cohort. This finding is compatible with the young and generally healthy profile of the participants and suggests a low burden of MRSA in this specific population [26]. However, the lack of MRSA should not reduce the relevance of the findings, since resistance to other antimicrobial agents was frequent among both *S. aureus* and CoNS. In this context, the main contribution of the study is not the detection of MRSA, but the demonstration that the oral cavity can harbour diverse phenotypically resistant *Staphylococcus* populations, including CoNS, in healthy young adults, with carriage and resistance patterns comparable to those observed in the nasal cavity.

The most frequent resistance profiles involved gentamicin, amoxicillin, erythromycin, and clindamycin. Resistance to amoxicillin, erythromycin, and clindamycin may reflect antimicrobial exposure patterns relevant to dental practice in Portugal [27], whereas gentamicin resistance suggests a broader AMR burden beyond dental prescribing. The interpretation of these results requires caution, particularly for amoxicillin, because species-specific EUCAST interpretive criteria are not available for several *Staphylococcus* spp. and the analysis was, therefore, exploratory. Nevertheless, the inclusion of amoxicillin is clinically relevant in dentistry, given its frequent use in oral healthcare, often alone or combined with clavulanic acid. The widespread use of beta-lactams, as well as macrolides and clindamycin as alternatives in patients reporting penicillin allergy, may contribute to selective pressure on oral microbial communities [12, 18, 19, 27, 28]. Thus, the observed resistance phenotypes reinforce the need for prudent

antibiotic prescribing in dentistry, although causal links between prescribing practices and resistance patterns cannot be inferred from this cross-sectional dataset.

The comparative analysis between oral and nasal samples further reinforces the need to consider the oral cavity in studies of staphylococcal carriage. For several species, including *S. epidermidis* and *S. aureus*, resistance and MDR frequencies did not differ significantly between oral and nasal carriers. These findings suggest that the oral cavity may be as relevant as the nasal cavity for detecting phenotypically resistant staphylococci. This is important because clinical screening and decolonization strategies for *S. aureus*, particularly MRSA, have traditionally focused on the anterior nares, whereas the oral cavity is less frequently considered [13, 14]. Although the present study does not evaluate decolonization outcomes, it supports the inclusion of oral sampling in future surveillance studies addressing staphylococcal carriage and AMR in community settings.

Longitudinal FTIR-ATR analysis provided exploratory evidence of species-specific colonization dynamics. For *S. warneri* the marked heterogeneity of spectral profiles across time points and the limited clustering of isolates from the same participant suggest a pattern compatible with temporal turnover. This may indicate that *S. warneri* behaves predominantly as a transient or unstable colonizer in the oral cavity under the conditions analysed. Such instability may reflect mechanical clearance by saliva, mastication, mucosal desquamation, oral hygiene practices, or competition with established members of the oral microbiota, particularly streptococci [4, 29, 30]. In contrast, *S. aureus* displayed more heterogeneous and complex evolutionary dynamics. The identification of subpopulations capable of prolonged persistence suggests that the mouth may act as a viable reservoir for this species, despite it not being a classic member of the primary oral microbiome [6, 13]. This ecological stability may reflect species-specific adaptive traits, which may include a greater capacity for epithelial adhesion, the invasion of specific niches, and integration into multi-species biofilms, enabling it to evade the local immune response and the normal mechanical clearance by saliva [15, 20, 31].

This study presents some limitations that should be acknowledged. The sample consisted exclusively of dental students from a single institution, which may limit the generalizability of the findings. Only a subset of 31 participants was followed longitudinally, reducing the robustness of conclusions regarding persistent versus transient colonization. Furthermore, the strain-level analysis was restricted to *S. aureus* and *S. warneri* to ensure both clinical relevance and analytical feasibility. *S. aureus* was selected due to its well-established clinical importance, while *S. warneri* was included as a representative coagulase-negative *Staphylococcus* species with sufficient isolate numbers to enable longitudinal analysis. Other species, such as *S. epidermidis*, were not included due to the high number of isolates, which exceeded the practical capacity for FTIR-based analysis within the scope of this study. Additionally, the methodology relied on the selection of specific colonies from culture samples rather than metagenomic sequencing, meaning it does not capture the complete profile of the bacterial community or the full within-sample strain diversity. Moreover, antimicrobial resistance was assessed phenotypically, without molecular characterization of resistance genes, mobile genetic elements, or clonal lineages. Therefore, the study cannot determine the genetic mechanisms underlying resistance or directly assess horizontal gene transfer. Despite these limitations, the results support the oral cavity as a relevant complementary niche for the carriage of phenotypically resistant *Staphylococcus* species, highlighting its potential value for future surveillance studies in community settings.

5. Conclusion

This study supports the oral cavity as a frequently colonized niche for *Staphylococcus* species, extending beyond the traditional view of a purely transient site. The high colonization rate and substantial oro-nasal overlap suggest that the oral cavity may represent a complementary ecological niche for staphylococcal carriage, independent of oral health status in this cohort. Notably, oral colonization was associated with phenotypic antimicrobial resistance, particularly

among coagulase-negative staphylococci, underscoring the importance of prudent antibiotic use and continued surveillance. Longitudinal FTIR-ATR analysis further suggested species-specific colonization dynamics, with transient patterns observed for *S. warneri* and indications of persistence of similar strain profiles for *S. aureus* in a subset of individuals. Future studies using larger cohorts and genomic approaches are needed to confirm strain persistence and clarify the mechanisms underlying antimicrobial resistance in oral staphylococci.

Declarations

Funding

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• Conflicts of Interest

The authors declare no conflicts of interest.

• Ethics approval

The study was approved by the Ethics Committee of the Faculty of Dental Medicine of the University of Porto (FMDUP) (Project No. 26/2019 and 19/2025). The study was conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

• Consent to participate

Written informed consent was obtained from all individual participants included in the study.

• Data Availability Statement

The data supporting the findings of this study are provided as Supplementary Material ("Supplementary Data") accompanying this article. Additional information may be available from the corresponding author upon reasonable request.

Author Contribution

Maeva Fonseca: Contributed to methodology, drafted the manuscript, and critically revised the manuscript.Marta Sousa: Contributed to methodology, drafted the manuscript, and critically revised the manuscript.Carla Campos: Contributed to methodology and critically revised the manuscript.Joana Campos: Contributed to methodology and critically revised the manuscript.Carolina F.F.A. Costa: Contributed to project conception, methodology, and critically revised the manuscript.Benedita Sampaio-Maia: Contributed to project conception and critically revised the manuscript.All authors: Read and approved the final manuscript.All authors gave their final approval and agree to be accountable for all aspects of the work.

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Data Availability

The data supporting the findings of this study are provided as Supplementary Material ("Supplementary Data") accompanying this article. Additional information may be available from the corresponding author upon reasonable request.

References

1. Krishnan K, Chen T, Paster BJ (2017) A practical guide to the oral microbiome and its relation to health and disease. Oral Dis 23(3):276–286. 10.1111/odi.12509
2. Baker JL, Mark Welch JL, Kauffman KM, McLean JS, He X (2024) The oral microbiome: diversity, biogeography and human health. Nat Rev Microbiol 22(2):89–104. 10.1038/s41579-023-00963-6
3. Azevedo MJ, Kaan AM, Costa C, Sampaio-Maia B, Zaura E (2025) Acquisition of the Oral Microbiome. Adv Exp Med Biol 1472:13–29. 10.1007/978-3-031-79146-8_2
4. Smith AJ, Jackson MS, Bagg J (2001) The ecology of Staphylococcus species in the oral cavity. J Med Microbiol 50(11):940–946. 10.1099/0022-1317-50-11-940
5. Hirose M, Aung MS, Fujita Y, Sakakibara S, Minowa-Suzuki E, Otomo M et al (2024) Prevalence, genetic characteristics, and antimicrobial resistance of staphylococcal isolates from oral cavity and skin surface of healthy individuals in northern Japan. J Infect Public Health 17(8):102488. 10.1016/j.jiph.2024.102488
6. Campos J, Pires MF, Sousa M, Campos C, da Costa C, Sampaio-Maia B (2023) Unveiling the Relevance of the Oral Cavity as a Staphylococcus aureus Colonization Site and Potential Source of Antimicrobial Resistance. Pathogens 12(6). 10.3390/pathogens12060765
7. Fumio Nagahama OT, Uchibori S, Fukumoto M (2013) Frequency of Staphylococci in Oral Cavity of Healthy Medical Workers. Int J Oral-Medical Sci 12:35–40
8. Madhaiyan M, Wirth JS, Saravanan VS (2020) Phylogenomic analyses of the Staphylococcaceae family suggest the reclassification of five species within the genus Staphylococcus as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five Staphylococcus species to Mammaliicoccus gen. nov., and the formal assignment of Nosocomiicoccus to the family Staphylococcaceae. Int J Syst Evol Microbiol 70(11):5926–5936. 10.1099/ijsem.0.004498
9. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O (2018) Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol 9:2419. 10.3389/fmicb.2018.02419
10. Raineri EJM, Altulea D, van Dijl JM (2022) Staphylococcal trafficking and infection-from 'nose to gut' and back. FEMS Microbiol Rev 46(1). 10.1093/femsre/fuab041
11. Göker M, Christensen H, Fingerle V, Kostovski M, Margos G, Moore ERB et al (2025) List of Recommended Names for bacteria of medical importance: report of the Ad Hoc Committee on Mitigating Changes in Prokaryotic Nomenclature. Int J Syst Evol Microbiol 75(10). 10.1099/ijsem.0.006943
12. Donkor ES, Kotey FC (2020) Methicillin-Resistant Staphylococcus aureus in the Oral Cavity: Implications for Antibiotic Prophylaxis and Surveillance. Infect Dis (Auckl) 13:1178633720976581. 10.1177/1178633720976581
13. Chmielewski M, Załachowska O, Komandera D, Albert A, Wierzbowska M, Kwapisz E et al (2024) The Oral Cavity-Another Reservoir of Antimicrobial-Resistant Staphylococcus aureus? Antibiotics. (Basel) 13(7). 10.3390/antibiotics13070649

14. Kearney A, Kinnevey P, Shore A, Earls M, Poovelikunnel TT, Brennan G et al (2020) The oral cavity revealed as a significant reservoir of Staphylococcus aureus in an acute hospital by extensive patient, healthcare worker and environmental sampling. J Hosp Infect. 10.1016/j.jhin.2020.03.004
15. Schilcher K, Horswill AR (2020) Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies. Microbiol Mol Biol Rev 84(3). 10.1128/mmbr.00026-19
16. Heilmann C, Ziebuhr W, Becker K (2019) Are coagulase-negative staphylococci virulent? Clin Microbiol Infect 25(9):1071–1080. 10.1016/j.cmi.2018.11.012
17. Silvola J, Gröndahl-Yli-Hannuksela K, Hirvioja T, Rantakokko-Jalava K, Kanerva M, Auranen K et al (2025) Increasing trend of antimicrobial resistance among methicillin-resistant Staphylococcus aureus strains in Southwest Finland, 2007–2016: An analysis of shifting strain dynamics and emerging risk factors. J Glob Antimicrob Resist 40:47–52. 10.1016/j.jgar.2024.11.015
18. Sharma S, Chauhan A, Ranjan A, Mathkor DM, Haque S, Ramniwas S et al (2024) Emerging challenges in antimicrobial resistance: implications for pathogenic microorganisms, novel antibiotics, and their impact on sustainability. Front Microbiol 15:1403168. 10.3389/fmicb.2024.1403168
19. Mancuso G, Midiri A, Gerace E, Biondo C (2021) Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens 10(10). 10.3390/pathogens10101310
20. Idrees M, Sawant S, Karodia N, Rahman A (2021) Staphylococcus aureus Biofilm: Morphology, Genetics, Pathogenesis and Treatment Strategies. Int J Environ Res Public Health 18(14). 10.3390/ijerph18147602
21. Roberts AP, Mullany P (2010) Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance. Expert Rev Anti Infect Ther 8(12):1441–1450. 10.1586/eri.10.106
22. Fišarová L, Botka T, Du X, Mašlaňová I, Bárdy P, Pantůček R et al (2021) Staphylococcus epidermidis Phages Transduce Antimicrobial Resistance Plasmids and Mobilize Chromosomal Islands. mSphere 6(3). 10.1128/mSphere.00223-21
23. Rybak B, Werbowy O, Debowski K, Plotka M, Kocot AM (2025) Coagulase-Negative Staphylococci Determined as Blood Culture Contamination Have High Virulence Characteristic Including Transfer of Antibiotic Resistance Determinants to Staphylococcus aureus and Escherichia coli. Int J Mol Sci 26(9). 10.3390/ijms26094424
24. Pal M, Rebuma T, Regassa T, Zende R (2024) Methicillin-Resistant Staphylococcus Aureus (Mrsa) Remains a Major Threat to Public Health. Am J Public Health Res 12(3):48–53. 10.12691/ajphr-12-3-2
25. Yue H, Shen P, Huang Q, Lin S, Shen J, Wang Y et al (2025) Associations Between Dietary Habits, Oral Microbiota, and Dental Caries in Adolescents. Phenomics 5(6):633–645. 10.1007/s43657-024-00208-3
26. Wielders CL, Fluit AC, Brisse S, Verhoef J, Schmitz FJ (2002) mecA gene is widely disseminated in Staphylococcus aureus population. J Clin Microbiol 40(11):3970–3975. 10.1128/jcm.40.11.3970-3975.2002
27. Saúde D-G (2014) Norma 010/2014 - Prescrição de Antibióticos em Patologia Dentária. Direção-Geral da Saúde
28. Albuquerque I, Melo P, Azevedo Á (2025) Antibiotic prescription habits of Portuguese oral healthcare professionals: an exploratory study. Revista Portuguesa de Estomatologia Med Dentária e Cirurgia Maxilofacial 66(3):130–137. 10.24873/j.rpemd.2025.09.1434
29. Akimbekov NS, Digel I, Yerezhepov AY, Shardarbek RS, Wu X, Zha J (2022) Nutritional factors influencing microbiota-mediated colonization resistance of the oral cavity: A literature review. Front Nutr 9:1029324. 10.3389/fnut.2022.1029324
30. Sampaio-Maia B, Caldas IM, Pereira ML, Pérez-Mongiovi D, Araujo R (2016) The Oral Microbiome in Health and Its Implication in Oral and Systemic Diseases. Adv Appl Microbiol 97:171–210. 10.1016/bs.aambs.2016.08.002
31. Mosaddad SA, Tahmasebi E, Yazdanian A, Rezvani MB, Seifalian A, Yazdanian M et al (2019) Oral microbial biofilms: an update. Eur J Clin Microbiol Infect Dis 38(11):2005–2019. 10.1007/s10096-019-03641-9

Figures

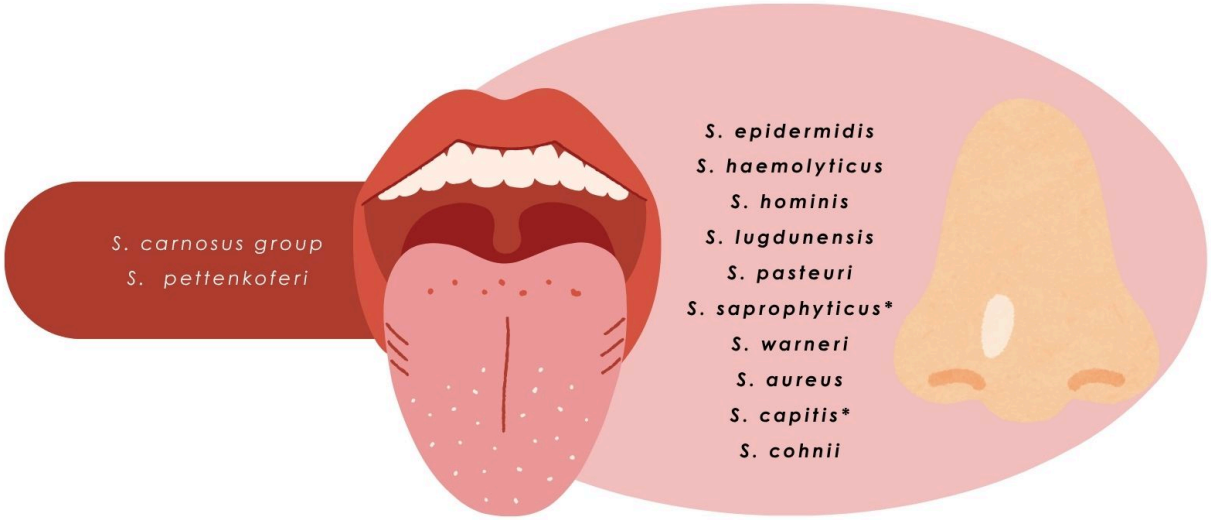


Figure 1

Schematic representation of *Staphylococcus* species detected exclusively in the oral cavity (left) and detected in both oral and nasal cavities (central area). Asterisks (*) indicate species with a statistically significant difference in colonization frequencies between paired oral and nasal cavity samples ($p < 0.05$) based on the exact McNemar's test.

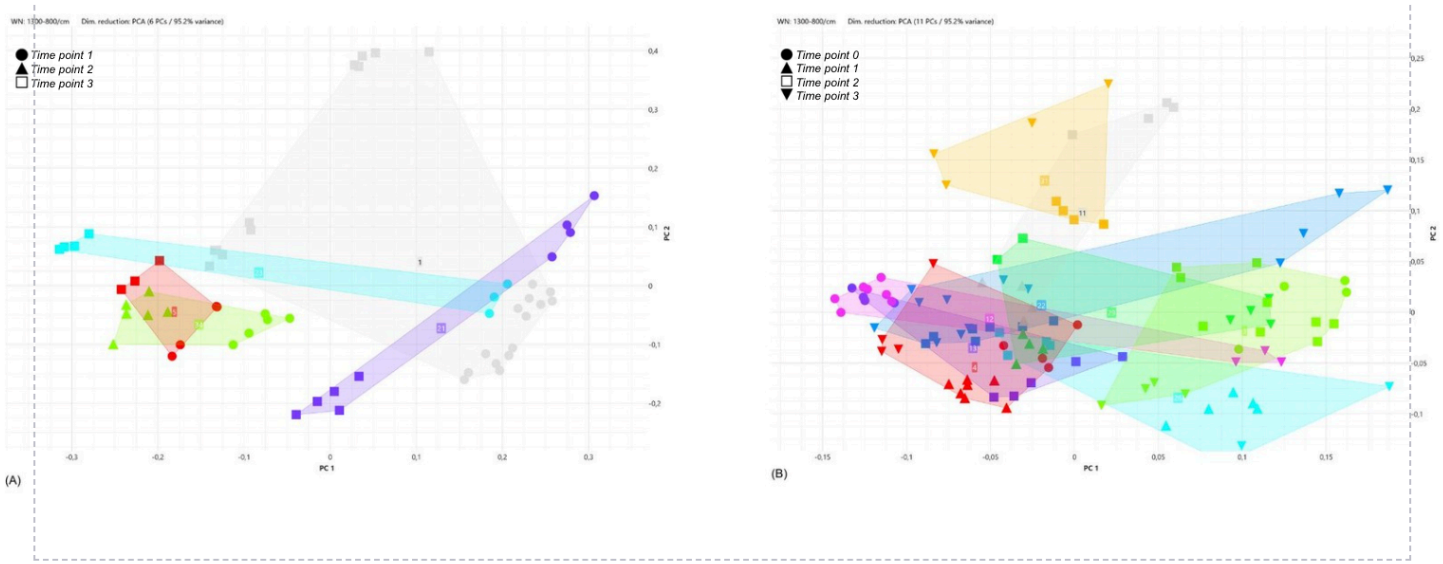


Figure 2

Scatter plots resulting from dimensionality reduction via Principal Component Analysis (PCA) for staphylococcal isolates across up to four sampling time points (T0 to T3). (A) *Staphylococcus warneri* spectral profiles (6 Principal Components explaining 95.2% of the variance), showing marked heterogeneity and reflecting temporal turnover. (B) *Staphylococcus aureus* spectral profiles (11 Principal Components explaining 95.2% of the variance), displaying persistent clustering profiles for a subset of participants alongside turnover for others. Each unique color corresponds to a specific participant, and each geometric shape represents a distinct collection time point. Time point 0 (●); Time point 1 (▲); Time Point 2 (□); Time point 3 (▼).

Supplementary Files

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