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ORIGINAL ARTICLE

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Presence and implication of *Candida* spp. in patients with peri-implantitis enrolled in a supportive peri-implant therapy program of the Basque Country (Spain). A case–control study

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Abstract

Introduction: The peri-implant sulcus is a good niche for infectious colonization such as *Candida* spp. In this study, the level of *Candida* spp. fungal colonization is analyzed in patients with peri-implantitis under supportive peri-implant therapy, as well as its correlation with the main clinicopathological data.

Methods: A case–control study was carried out on 161 patients treated with dental implants, 80 with PI and 81 without PI, which corresponded to 91 women and 70 men, whose mean age was 60.90 years. A specific protocol was completed for the clinical and implant data. Microbiological samples were taken by oral rinse and with paper tips from the peri-implant sulcus. For the quantitative and qualitative analysis *Candida* Chromogenic Agar/CONDA plates were incubated for 72 h at 36 + 1°C. Fungal growth was considered active when having more than 50 CFU. Specific *Candida* spp. cultures were later confirmed by API ID 32C and PCR.

Results: Fungal growth was achieved in 28% of oral rinse and 6.75% of peri-implant fluid samples. No significant differences were recognized between study groups. Most of the cultures (>65%) showed more than 50 CFU. The most frequent species were *Candida albicans* and *Candida parapsilosis*. There was no association between different PI risk factors and fungal data. The presence of *Candida* spp. in the oral cavity of patients with dental implants was related to total edentulism and the use of implant-fixed complete prosthesis implant-retained removable prosthesis.

Conclusions: These results suggest that there is no link between PI and presence of *Candida* in patients with dental implants undergoing regular supportive periodontal therapy.

KEYWORDS

candida spp., case–control, peri-implantitis, supportive peri-implant therapy program

What is known

- Presence of *Candida* spp. on the peri-implant fluid surrounding dental implants has long been discovered.
- None of studies performed to date on the relationship between *Candida* colonization and peri-implantitis have related the fungal findings with key clinical data.

What this study adds

- This study proves there is no difference on the presence of *Candida* in the peri-implant fluid of healthy dental implants and those diagnosed with peri-implantitis, in patients under supportive peri-implant therapy program.
- Colonization of *Candida* spp. in the oral cavity of patients with dental implants is higher among patients with implant-fixed complete prosthesis or implant-retained removable prosthesis.

1 | INTRODUCTION

Peri-implantitis (PI) is a multifactorial disorder that affects up to 50% of patients with dental implants and causes progressive loss of supporting bone, leading in many cases to treatment failure.[1] PI is described as a polymicrobial infectious disease involving different infectious agents, mainly bacterial (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, etc.).[2–4]

Other agents like *Candida* spp. could also be involved in PI, as the peri-implant sulcus is a good niche for fungal colonization.[5–7] Due to the anaerobic environment of the peri-implant pocket, *Candida* spp. could be implicated in peri-implant bone loss, while promoting its virulence via increase of Sap activity, maintaining the inflammatory response and attracting other peri-implant pathogens.[8]

A recent systematic review conducted by our group about the involvement of this fungus in peri-implant disease[9] recognized that *Candida spp*. is present in the peri-implant fluid of both healthy implants (9%–50%) and those with PI (3%–76%); but with no statistically significant differences. Previous studies on this pathogenic role have not assessed important factors such as the number of colony-forming units (CFU), the probing depth (PD) of the implants, marginal bone loss, or the type of implant-supported prosthesis; nor have they performed a correct clinicomycological characterization, comparing implants with and without PI.[9] In addition, no studies have yet been made with PI-patients under control, which could be an important factor in this possible association.

With this background, we proposed to conduct a case–control study based on the hypothesis that fungi of the genus *Candida* spp. would actively participate in the etiopathogenesis and maintenance of peri-implantitis. The main objective of our study was to acknowledge the colonization level of *Candida* and its species in patients of the Basque Country (Spain) with peri-implantitis under regular supportive peri-implant therapy (SPT) program, as well as its association with the main clinicopathological data of this disorder.

2 | METHODS

2.1 | Patients

A case–control study was conducted on 161 patients following a regular SPT program from the Periodontics and Osseointegration Unit of the Dental Clinic Service of the University of the Basque Country (Leioa, Spain) and the COMQ Centre (Barakaldo, Spain).

This Project follows the principles of the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, and has been approved by the UPV/EHU Research Ethics Committee (CEISH: M10/2016/057, CEIAB/2016/180). The current study matches STROBE guidelines, and all participants signed an informed consent form before participating in the study.

Inclusion criteria for patients were: (1) being over 18 years old, (2) having at least one dental implant in function for a minimum time of 1 year, and (3) being enrolled in an individualized SPT program.[10]

Exclusion criteria for patients were: (1) having received periodontal tissue healing, antibiotics and/or bone metabolism-related drugs during the last 6 months prior to mycological sampling, (2) having cement-retained implant-supported dental restorations, and (3) having been surgically treated for peri-implantitis in the last 6 months.

Participants included in the Case Group (CA) were diagnosed with PI on at least one dental implant, according to the criteria by Renvert et al.[11]: (1) Evidence of visual inflammatory changes in the peri-implant soft tissues combined with bleeding on probing and/or suppuration; (2) Increasing probing pocket depths as compared to measurements obtained at placement of the supra-structure; (3) Progressive bone loss in relation to the radiographic bone level assessment at 1 year following the delivery of the implant-supported prosthetics reconstruction; (4) In the absence of initial radiographs and probing depths, radiographic evidence of bone level ≥3 mm and/or probing depths ≥6 mm in conjunction with profuse bleeding represents peri-implantitis.

Participants included in the Control Group (CO) only had healthy implants without PI.

The SPT program in the CO group consisted on: (A) Mechanical cleansing using rubber cups and polishing paste, acrylic scalers for chipping off calculus; instruction for more effective oral hygiene practices. The SPT program in the CA group consisted on: (A + B) Antiseptic therapy. Rinses with 0.1%–0.2% chlorhexidine digluconate for 30 s using approximately 10 mL.

All the included patients were treated with threaded and modified surface titanium dental implants: Straumann® (Basel, Switzerland), AstraTech® (Molndal, Sweden), Ticare Mozo Grau® (Valladolid, Spain), or Nobel Biocare® (Kloten, Switzerland).

A specific clinicopathological protocol was completed in all cases, which gathered general and medical characteristics of the patients: age, gender, follow-up time, smoking habit (more or less than 10 cigarettes per day; ex-smokers for less than 10 years), alcohol consumption (number of alcoholic units per week), systemic diseases, history of periodontitis (aggressive, chronic),[12] edentulism (partial or total) and prosthetic rehabilitation (single-unit implant restoration, multi-unit implant restoration, implant-fixed complete prosthesis, implant-retained removable prosthesis).

The clinical implant data included: number and location, PD, bleeding (BOP) and/ or suppuration on probing, and marginal bone loss (MBL) (through periapical intraoral radiographs with parallel technique at the time of mycological sampling).

2.2 | Mycological sampling

Two types of mycological samples were taken in all patients: (1) Oral rinse with 20 mL of sterile milli-Q water for 1 min, and (2) Sterile paper tips in the peri-implant sulcus for 30 s. For the peri-implant fluid sample, this was obtained from a healthy implant in the CO, and from a healthy implant (if any) and an implant with PI (always from the implant with the highest marginal bone loss) in the CA.

For the analysis, 100 μL of the oral rinse (pellet) and 50 μL of the paper tip sample were collected, spread and cultured at 36 + 1°C on *Candida* Chromogenic Agar/CONDA plates (CondaLab®, Madrid, Spain). Mycological growth was assessed at 24, 48, and 72 h, after which quantitative (CFU) and qualitative analysis of the colonies (color, shape, and texture) were performed.

The isolated species were finally confirmed by API ID 32C® (bioMérioux, Marcy L'Etoile, France) and PCR techniques.[13]

API ID 32C is a carbon source assimilation test, composed of 32 domes: 29 with a dehydrated carbon substrate, 1 negative control, 1 sensitive to chloheximide, and 1 colorimetric test for esculin. This technique allows the identification of 63 different species of yeast organisms: *Candida albicans*, *Candida boidinii*, *Candida catenulata*, *Candida colliculosa*, *Candida dattila*, *Candida dubliniensis*, *Candida famata*, *Candida glabrata*, *Candida globosa*, *Candida guilliermondii*, *Candida hellenica*, *Candida holmii*, *Candida inconspicua/norvegensis*, *Candida intermedia*, *Candida kefyr*, *Candida krusei*, *Candida lambica*, *Candida lipolytica*, *Candida lusitaniae*, *Candida magnoliae*, *Candida melibiosica*, *Candida membranifaciens*, *Candida norvegica*, *Candida parapsilosis*, *Candida pelliculosa*, *Candida pulcherrima*, *Candida rugosa*, *Candida sake*, *Candida silvicola*, *Candida sphaerica*, *Candida tropicalisy*, *Candida utilis*, *Candida valida*, *Candida zeylanoides*, *Cryptococcus albidus*, *Cryptococcus curvatus*, *Cryptococcus humicola*, *Cryptococcus laurentii*, *Cryptococcus neoformans*, *Cryptococcus terreus*, *Cryptococcus uniguttulatus*, *Debaryomyces etchellsii/carsonii*, *Debaryomyces polymorphus*, *Geotrichum capitatum*, *Geotrichum* spp., *Kloeckera apis/apiculata*, *Kloeckera japonica*, *Kodamaea ohmeri*, *Pichia farinosa*, *Rhodotorula glutinis*, *Rhodotorula minuta*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae Saccharomyces kluyverii*, *Sporobolomyces salmonicolor*, *Stephanoascus ciferrii*, *Trichosporon inkin*, *Trichosporon asahii*, *Trichosporon mucoides*, *Williopsis saturnus Zygosaccharomyces* spp.

For the final discrimination between *Candida africana*, *C. dubliniensis* and *C. albicans*, PCR (CR-f/CR-r) with different hwp1 gene amplifications were performed: 700 pb for *C. albicans*, 569 pb for *C. dubliniensis* and 941 pb for *C. africana*.[13]

2.3 | Statistical analysis

Qualitative variables were described with frequencies and percentages; and quantitative variables with mean, standard deviation, and ranges. For the bivariate analysis, Chi-square test was used if the variables were categorical, and Student's *t*-test when having a quantitative variable and a qualitative variable of two categories. To analyze the risk, binary logistic regressions were performed.

It was considered statistically significant when $p < 0,05$. IBM® SPSS® v.28 statistical software was used for all analyses.

3 | RESULTS

3.1 | Patients

The main data of the 161 patients are featured in Table 1. In total, 91 women and 70 men were included, with a mean age of 60.90 ± 10.22 years at the time of the study. There were no significant differences between the study groups in relation to gender and age. The mean evolution until diagnosis of PI was 4.61 ± 2.50 years; with the clinical follow-up time being longer in the CA ($p < 0.01$) (Table 1).

The number of patients who smoked was significantly higher in the CA ($p < 0.05$). There were not strong differences in ex-smokers or alcohol consumers among both study groups (Table 1). High blood-pressure was the most frequent systemic pathology, followed by hypercholesterolemia, depression, and hypothyroidism. The percentage of patients with diabetes mellitus was low (Table 1).

No statistically significant differences were seen in relation to history of periodontitis, either aggressive or chronic, between patients in the CA and the CO ($p = 0.38$) (Table 1). Same to the type of edentulism or its location. Regarding the type of prosthetic rehabilitation, patients with PI had more multi-unit implant restorations ($p < 0.05$) (Table 1).

TABLE 1 General and clinical data of the patients included in the study.

General data	Study group			p
	CA (*n*: 80)	CO (*n*: 81)	Total (*n*: 161)	
Gender: *n* (%)				
• Female	43 (53.80)	48 (59.30)	91 (56.50)	0.58
• Male	37 (46.30)	33 (40.70)	70 (43.50)	
Age (years)				
• Mean ± SD	61.23 ± 8.92	60.70 ± 11.41	60.90 ± 10.22	0.37
• Range	(37–77)	(31–86)	(31–86)	
Follow-up time (years)				
• Mean ± SD	7.45 ± 3.19	5.41 ± 3.14	6.43 + 3.32	**<0.01**
• Range	(2–13)	(1–13)	(1–13)	
Tobacco consumption: *n* (%)				
• Non-smoker	22 (27.50)	40 (49.40)	62 (38.50)	**<0.05**
• Smoker <10 cig/day	4 (5)	5 (6.20)	9 (5.60)	
• Smoker ≥10 cig/day	17 (21.40)	6 (7.40)	23 (14.20)	
• Ex-smoker <10 cig/day	2 (5.40)	3 (10)	5 (7.50)	0.65
• Ex-smoker ≥10 cig/day	35 (94.60)	27 (90)	62 (92.50)	
Alcohol consumption: *n* (%)				
• None	42 (53.50)	42 (51.90)	84 (52.20)	0.85
• <7 U/week	20 (25)	23 (28.40)	43 (26.70)	
• ≥7 U/week	18 (22.50)	16 (19.80)	34 (21.10)	
Systemic pathology: *n* (%)				
• High-blood pressure	22 (27.50)	25 (30.90)	47 (29.20)	0.22
• Hypercholesterolemia	12 (15)	13 (16)	25 (15.50)	0.85
• Depression	4 (5)	9 (11.10)	13 (8.10)	2.03
• Hypothyroidism	7 (8.80)	5 (6.20)	12 (7.50)	0.39
• Asthma	6 (7.50)	6 (6.20)	11 (6.80)	0.11
• Diabetes mellitus II	4 (5)	6 (7.40)	10 (6.20)	0.39
• Cardiovascular disease	4 (5)	6 (7.60)	10 (6.20)	0.33
• Cancer	4 (5)	5 (6.2)	9 (5.60)	0.10
• Hiatal hernia	3 (3.8)	5 (6.2)	8 (5)	0.19
History of periodontitis: *n* (%)				
• Si	34 (42.50)	29 (35.80)	63 (39.10)	0.38
• No	46 (57.50)	52 (64.20)	98 (60.90)	
Edentulism: *n* (%)				
• Total	12 (15)	11 (13.60)	23 (14.30)	0.39
• Anterior	5 (7.60)	2 (3.10)	7 (5.40)	021
• Posterior	20 (31.30)	29 (43.90)	49 (37.70)	0.54
• Upper maxilla	25 (37.90)	19 (29.70)	44 (33.80)	0.97
• Mandible	25 (37.90)	16 (25)	41 (31.50)	0.11
Prosthetic rehabilitation: *n* (%)				
• Crown	26 (34.70)	37 (49.30)	63 (42)	0.07
• Crown and bridges	6 (7.40)	5 (6.30)	11 (6.80)	0.77
• Bridges	36 (48)	24 (32)	60 (40)	**<0.05**
• Hybrid prosthesis	16 (20)	12 (14.80)	28 (18.70)	0.39
• Overdenture	0 (0)	4 (4.90)	4 (2.50)	0.13

Note: Bold values indicate correspond to statistically significant results.
Abbreviations: CA, case group; CO, control group; SD, standard deviation.

TABLE 2 Clinical data of the dental implants analyzed in the study.

	Study group			
Data	**CA**	**CO**	**Total**	***p***
Implants (*n*)	418	381	799	0.17
Peri-implant diagnosis: *n* (%)				
• Peri-implantitis	229 (54.78)	0 (0)	229 (28.66)	–
• Health	189 (45.22)	381 (100)	570 (71.34)	
Data	**CA with PI (*n*: 229)**	**Healthy CA and CO (*n*: 570)**	**Total (*n*: 799)**	***p***
Location: *n* (%)				
• Anterior	68 (29.70)	167 (29.30)	229 (28.90)	0.71
• Posterior	161 (70.30)	403 (70.70)	564 (71.10)	
• Upper maxilla	87 (38)	221 (38.80)	318 (39.80)	0.68
• Mandible	142 (62)	349 (61.20)	481 (60.20)	
PD (mm)				
• Media ± SD	5.13 ± 1.25	1.72 ± 1.00	3.41 ± 1.96	**<0.01**
Marginal bone loss (mm)				
• Media ± SD	5.58 ± 1.13	2.42 ± 1.18	3.99 ± 2.05	**<0.01**
BOP: *n* (%)				
• Si	129 (56.30)	31 (5.40)	160 (20)	**<0.01**
• No	100 (43.80)	539 (94.60)	639 (80)	
Suppuration: *n* (%)				
• Yes	32 (14)	0 (0)	32 (4)	**<0.01**
• No	197 (86)	570 (100)	767 (96)	

Note: Bold values indicate correspond to statistically significant results.
Abbreviations: BOP, bleeding on probing; CA, case group; CO, control group; PD, probing depth; PI, peri-implantitis; SD, standard deviation.

3.2 | Implants

The data of the 799 analyzed dental implants appear in Table 2, out of which 229 were diagnosed with PI. Overall, a higher number of dental implants were located in the posterior area of the mandible, with no significant differences between study groups (Table 2).

Dental implants with PI showed bigger PD and MBL marginal bone loss, and presence of BOP and suppuration was more common ($p < 0.01$) (Table 2).

3.3 | Mycology

3.3.1 | Oral rinse

The results of the 161 oral rinse samples are shown in Table 3. Fungal growth was obtained in 28% of the samples, most of them with more than 50 CFU, but with no significant differences between both study groups. No patient had clinical evidence of oral candidiasis at the time of mycological sampling.

Most of the cultures obtained were simple (one microorganism), with *C. parapsilosis*, *C. guillermondi*, and *C. albicans* being the most frequently isolated species in the CA; and *C. albicans*, *C. parapsilosis*, and *C. glabrata* in the CO (Figure 1). Other fungal forms were also recognized (Table 3).

Mixed cultures corresponded mainly to *C. parapsilosis* + *C. guillermondi*, *C. parapsilosis* + *C. famata*, *C. lipolytica* + *C. albicans* and *C. zeylanoides* + *C. lipolytica* (Figure 1).

No general data of patients could be related to the mycological results. However, a statistically significant association was observed between the growth of *Candida spp*. and CO group patients with total edentulism ($p = 0.04$), and using implant-fixed complete prosthesis ($p = 0.03$) or implant-retained removable prosthesis ($p = 0.05$) (Table 4).

3.3.2 | Peri-implant fluid

The findings of the 214 mycological samples obtained with paper tips are displayed in Table 5. Fungal growth was only observed in 10% of implants with PI and 4.20% of healthy-implants, with no significant differences. Most fungal growths, of both CA and CO, showed more than 50 CFU.

All cases were single cultures, mostly corresponding to *C. albicans* and *C. parapsilosis* in PI cases, and *C. parapsilosis* in healthy implants (Figure 1) (Table 5).

TABLE 3 Presumptive and final fungal identification of the oral rinse samples: comparative analysis.

	Study group			
Mycological data	**CA: *n* (%)**	**CO: *n* (%)**	**Total: *n* (%)**	***p***
Fungal growth				
(+)	20 (25)	25 (30.90)	45 (28)	0.48
(−)	60 (75)	56 (69.10)	116 (72)	
CFU				
>50	14 (70)	15 (60)	31 (68.90)	0.54
<50	6 (30)	8 (40)	14 (31.10)	
Culture				
Simple	15 (75)	23 (92)	38 (84.40)	0.21
Mixed	5 (25)	2 (8)	7 (15.60)	
Candida species				
C. albicans	4 (20)	7 (28)	11 (24.40)	>0.05
C. parapsilosis	8 (40)	4 (17.40)	12 (26.70)	
C. guillermondi	5 (25)	2 (8)	7 (15.60)	
C. lipolytica	3 (12)	2 (8)	5 (11.10)	
C. zeylanoides	0 (0)	3 (12)	3 (6.70)	
C. glabrata	0 (0)	3 (12)	3 (6.70)	
C. krusei	0 (0)	1 (4)	1 (2.20)	
C. pelliculosa	0 (0)	1 (4)	1 (2.20)	
C. incons. norvengen	0 (0)	1 (4)	1 (2.20)	
C. famata	0 (0)	1 (4)	1 (2.20)	
Other fungal species				
Debaryomyces etch. carso	1 (6.70)	1 (4)	2 (4)	>0.05
Geotrichum spp.	1 (6.70)	0 (0)	1 (2)	
Debaryomyces polymorphus	0 (0)	1 (4)	1 (2)	

Abbreviations: CA, case group; CFU, colony-forming unit; CO, control group.

No association was recognized between the growth of *Candida* spp. in the peri-implant fluid samples and those obtained by oral rinse. No link of *Candida* growth with general and implant data was found either.

4 | DISCUSSION

Peri-implantitis is a common multifactorial disorder that develops in patients treated with dental implants of the Basque Country (Spain), as recognized by other authors in other populations.[14]

Poor plaque control and/or lack of regular SPT program, together with tobacco consumption and a history of periodontitis are the main risk factors for PI.[1,15,16] In our study, all patients were enrolled in a regular SPT program and had good plaque control (<25%).

In relation to tobacco, there was a significant relationship in the CA between smoking more than 10 cig/day and the presence of PI, similar to that previously reported in other studies.[17,18] Nonetheless, no direct relationship between the existence of PI and history of periodontal disease was achieved.[19,20] It is known that PI risk in patients who do not attend regular SPT program can be even 14 times higher than that of well-controlled ones,[21,22] which could explain the results of our regularly monitored patients.

Although the infectious component of PI is mainly bacterial, the role of other common pathogens in the oral cavity, such as *Candida* spp. remains to be revealed, in both the genesis and the evolution of this disorder.[23]

Fungi of *Candida* genus include more than 100 species, most of which live in endosymbiosis with humans.[24] Oral *Candida* spp. infections (oral candidiasis) appear in patients with one or more facilitating factors that enable their growth (immunosuppression, hiposialia, use of drugs, etc.).[25] Moreover, about 80% of candidiasis are caused by *C. albicans*, although other species like *C. parapsilosis*, *C. glabrata*, *C. krusei*, etc., are increasingly becoming more prevalent.[26]

Candida spp. grow by forming biofilms of yeast cells that synthesize a complex three-dimensional extracellular matrix.[23,27,28] Inorganic surfaces such as dental implants provide a good niche for the formation of these biofilms.[6,29] The adhesion of *Candida* to titanium relies on different factors of the implant surface like roughness, free energy, certain chemical properties, etc.[30] Rough implant surfaces promote a firmer and faster osseointegration, but also favor bacterial and fungal colonization.[31–33]

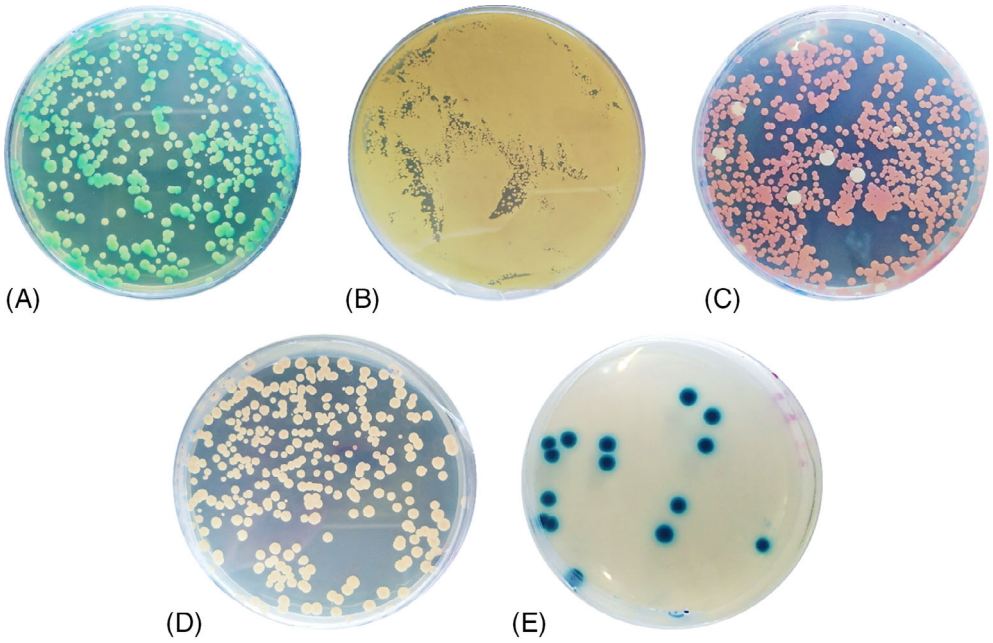

FIGURE 1 Single and mixed cultures of *Candida* spp. in the oral rinse and peri-implant fluid samples. *Candida* Chromogenic Agar/ CONDA plates. (A) Green CFU of *C. albicans*. (B) Brown-white CFU of *C. parapsilosis*. (C) White CFU of *C. lipolytica* and green CFU of *C. albicans*. (D) Brown-white CFU of *C. parapsilosis*. (E) Green CFU of *C. albicans*.

TABLE 4 Comparative analysis of oral rinse samples and clinical data.

	CO			CA		
	Mycological data: *n* (%)			Mycological data: *n* (%)		
	Fungal growth			Fungal growth		
Clinical data	**(+)**	**(−)**	***p***	**(+)**	**(−)**	***p***
Total edentulism						
No	18 (72)	52 (92.90)	**0.04**	52 (86.60)	70 (86.40)	0.72
Yes	7 (28)	4 (7.10)		8 (13.30)	11 (13.60)	
Overdenture						
No	22 (88)	55 (98.20)	**0.05**	20 (100)	80 (100)	–
Yes	3 (12)	1 (1.80)		0 (0)	0 (0)	
Hybrid-prosthesis						
No	18 (72)	51 (91.10)	**0.03**	16 (80)	48 (80)	0.64
Yes	7 (28)	5 (8.90)		4 (20)	12 (20)	
Crowns/bridges						
No	22 (88)	53 (94.60)	**0.29**	18 (90)	57 (95)	**0.79**
Yes	3 (12)	3 (5.40)		2 (10)	3 (5)	

Note: Bold values indicate correspond to statistically significant results.
Abbreviations: CA, case group; CFU, colony-forming unit; CO, control group.

Our study is the first to qualitatively and quantitatively analyze the presence of *Candida spp*. in oral and peri-implant fluid samples obtained from patients treated with dental implants enrolled in a regular SPT program.

Unfortunately, our oral rinse findings could not be compared with previous results, due to the lack of studies. However, the percentage of fungal growth (27.95%) was lower than in other studies performed in the same geographical region on periodontal patients with a similar age (48.50%).[34] This difference would be related to the SPT protocol of our patients.[35] Furthermore, this measure also explains the absence of significant differences in *Candida* spp. growth between patients in both study groups.

On the contrary, a significant association was found between colonization of *Candida* spp. and total edentulism, and the use of implant-fixed complete prosthesis and/ or implant-retained removable prosthesis, similar to Kilic et al.[36] In our opinion, *Candida* spp. would be independent of the existence of PI, and would thus be linked to these types of prosthetic rehabilitations, as it occurs in patients with

TABLE 5 Presumptive and final fungal identification of the peri-implant fluid samples: comparative analysis.

	Study group			
Mycological data	CA with PI: *n* (%)	Healthy CA and CO: *n* (%)	Total	*p*
Fungal growth				
(+)	8 (10)	6 (4.10)	14 (6.50)	0.84
(−)	62 (90)	138 (95.90)	200 (93.50)	
CFU				
>50	7 (87.50)	3 (50)	10 (71.40)	>0.05
<50	1 (12.50)	3 (50)	4 (28.60)	
Candida spp.				
C. albicans	3 (37.50)	0 (0)	3 (21.40)	0.18
C. parapsilosis	2 (25)	2 (33.30)	4 (28.70)	
C. lipolytica	2 (25)	0 (0)	2 (14.30)	
C. glabrata	1 (12.50)	2 (33.30)	3 (21.40)	
C. pelliculosa	0 (0)	1 (16.70)	1 (7.10)	
C. holmii	0 (0)	1 (16.70)	1 (7.100)	

Abbreviations: CA, case group; CFU, colony-forming unit; CO, control group; PI, peri-implantitis.

removable prostheses without dental implants.[25] It has been shown that the adhesion of *Candida* spp. relies, largely, on the prosthetic material[37]; its presence and permanence being greater in resins. Implant-fixed complete prostheses and full-mouth implant supported hybrid restorations, which contain bigger amounts of resin, would favor a stronger colonization by *Candida* spp. than bridges or single crowns. A majority of these patients showed more than 50 CFU on the culture, which can be considered as the borderline between colonization and fungal infection.[25] Therefore, when having an active *Candida* growth in patients with dental implants (>50 CFU), we propose the application of a preventive topical antifungal treatment with a 5-min non-alcoholic oral rinse of nystatin solution 100.000 UI/cc.

Although the peri-implant sulcus has been considered a good niche for fungal colonization,[6] the number of positive cultures was lower (7.63%) than those reported in previous studies.[1,3,38,39] Apart from the diagnostic PI criteria used in our study,[11] this difference could also be related to only having included patients under regular SPT program.[9] Furthermore, all the participants of our study only wore screw retained implant-supported dental prosthesis, which enables a better oral hygiene and peri-implant control.[40] Cement retained prostheses promote a bigger peri-implant mucosa inflammation and accumulation of different microbial agents, including fungal.[41]

The presence of *Candida* in the peri-implant fluid was higher in implants with PI than in healthy implants, although the differences were not significant, as reported in all studies performed so far.[5,36,40–42] The presence of more *Candida* cultures with more than 50 CFU in PI patients was not significant either. Moreover, it was impossible to relate the colonization of these fungi in the peri-implant fluid to any clinical implant data (PS, POM, BOP, and suppuration).

All these findings allow us to affirm that the existence of *Candida* spp. in the peri-implant fluid is not related to diagnosis of PI and/or its clinical parameters, as previously pointed.[9]

The most commonly identified *Candida* species was similar in both sample types, *C. albicans* and *C. parapsilosis*, which is similar with other studies.[5–7] An interesting result of our study was the high prevalence of *C. parapsilosis*. Although *C. parapsilosis* was initially described as a commensal yeast with no clinical relevance,[43] the increase in nosocomial infections and the use of medical devices have increased its incidence.[44] *C. parapsilosis* has a strong ability to form biofilms on different materials, including implant titanium surfaces, denture resins, etc.[45–49] The repeated use of antiseptic rinses with chlorhexidine could justify this high presence on these patients. Chlorhexidine is known to have an effective antifungal capacity against *C. albicans*,[50] further explaining its lower presence in our study, allowing the growth of other more resistant species such as *C. parapsilosis*.

In summary, our findings show the little clinical implication of *Candida* spp. in PI. However, patients who carry certain prosthetic rehabilitations (implant-fixed complete prosthesis, implant-retained removable prosthesis), or those with total edentulism, would have a higher risk to develop oral and peri-implant tissue candidiasis. In these cases, prevention and treatment with topical antifungals could be important to avoid *Candida* spp. colonization, aside from regular maintenance antiseptics.

The main limitation of our study was the impossibility to assess the presence of *Candida* spp. as a risk factor for PI, due to the small number of positive fungal growth cases. Therefore, our aim in the future is to increase the sample size and search for relationships related with *Candida* spp. in the oral cavity with important data such as diet, hyposialia, other oral microorganisms, etc. In addition, we are designing different studies to acknowledge the response to antifungal medication, used both therapeutically and preventively, in the clinicopathological evolution of PI.

5 | CONCLUSIONS

Based on our results, we can conclude that colonization of *Candida* fungi and its species in patients of the Basque Country with PI under SPT is

low, and does not play a relevant role in the peri-implant microbiota. Its presence would only be related to the type of prosthetic rehabilitation.

Because oral infection by *Candida* spp. show geographic variability, more prospective studies should be performed in the future, in order to determine its real pathogenic role in peri-implantitis, as well as other associated factors.

AUTHOR CONTRIBUTIONS

All listed have made substantial contributions to conception and design of the study. Irene Lafuente-Ibáñez-de-Mendoza and Rafael Martínez-Conde-Llamosas made substantial contributions to the acquisition of data; Irene Lafuente-Ibáñez-de-Mendoza and Xabier Marichalar-Mendia made substantial contributions to the analysis and interpretation of data; Irene Lafuente-Ibáñez-de-Mendoza and José Manuel Aguirre-Urizar drafted the work; Aitziber Fernández-Jiménez, Ana María García-De-La-Fuente, and José Manuel Aguirre-Urizar have made substantial contributions to revising the work. All listed authors have agreed to the final submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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