UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia

Dissertação

Estudo retrospectivo de candidíase oral, isolamento e identificação de variáveis associadas com candidíase atrófica crônica.

Leandro Calcagno Reinhardt

Pelotas, 2015

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RESUMO

REINHARDT, Leandro Calcagno. **Estudo retrospectivo de candidíase oral, isolamento e identificação de variáveis associadas com candidíase atrófica crônica.** 2015. 80 f. Dissertação de Mestrado. Programa de Pós-Graduação em Odontologia, Universidade Federal de Pelotas, Pelotas, 2015.

O tratamento de escolha para a candidíase atrófica crônica (estomatite por dentadura) é o uso de antifúngicos, geralmente na forma tópica, mas também a terapia sistêmica pode ser necessário em alguns casos. Seja de forma sistêmica ou tópica, o uso prolongado e recorrente dos antifúngicos contribui para o desenvolvimento de espécies resistentes. Este estudo teve como objetivo realizar o levantamento do perfil demográfico e clínico dos pacientes diagnosticados com candidíase e tratados no Centro de Diagnóstico de Doenças da Boca (CDDB), da Faculdade de Odontologia de Pelotas (FO/UFPel), no período de 1997 a 2014, e isolar, identificar e avaliar a suscetibilidade antifúngica de leveduras orais isoladas de pacientes diagnosticados com candidíase atrófica crônica (estomatite por dentadura) que apresentem resistência ao tratamento com o uso dos antifúngicos convencionais. Foram avaliados os prontuários dos pacientes atendidos no Serviço (CDDB) durante 18 anos. Variáveis como: raça, gênero, idade, doenças sistêmicas, tipo e localização da candidíase, sintomas e hábitos nocivos foram coletados. Além disso, foi realizada a coleta e o isolamento de *Candida* de pacientes com diagnóstico clínico de Candidíase atrófica crônica. Posteriormente, foi realizada a identificação das espécies e a avaliação de sua resistência antifúngica através do método automatizado Vitek 2 (Biomeriéux). A análise estatística foi realizada através dos testes qui-quadrado e Regressão de Poison. O nível de significância utilizado nos testes foi de 5% ($p \leq 0,05$). O software utilizado foi o STATA 13.1.

Palavras-chave: *Candida*; candidíase bucal; farmacorresistência fúngica; estomatite sob prótese.

ABSTRACT

REINHARDT, Leandro Calcagno. **Retrospective study of oral candidiasis, isolation and identification of variables associated with chronic atrophic candidiasis.** 2015. 80 f. Dissertação de Mestrado. Programa de Pós-Graduação em Odontologia, Universidade Federal de Pelotas, Pelotas, 2015.

The treatment of choice for chronic atrophic candidiasis (denture stomatitis) is the use of antifungals, usually in topical form but also systemic therapy may be required in some cases. Be systemic or topical form, prolonged and repeated use of antifungal contributes to the development of resistant strains. This study aimed to survey the demographic and clinical profile of patients diagnosed with candidiasis and treated in the Mouth Disease Diagnostic Center (CDDB), Faculty of Dentistry of Pelotas (FO / UFPel) in 1997 to 2014 and isolate, identify and evaluate the antifungal susceptibility isolated yeast oral candidiasis patients diagnosed with chronic atrophic (denture stomatitis) showing resistance to treatment with the use of conventional antifungal agents. We evaluated the records of patients seen in the Service (CDDB) for 18 years. Variables as race, gender, age, systemic diseases, type and location of candidiasis symptoms and harmful habits were collected. Furthermore, the collection and isolation of Candida from patients with a clinical diagnosis of chronic atrophic candidiasis was performed. Later, species identification and evaluation of their antifungal resistance through automated method Vitek 2 (bioMérieux) was performed. Statistical analysis was performed using the chi-square test and Poison Regression. The significance level used in the tests was 5% ($p \leq 0.05$). The software being used was the STATA 13.1.

Keywords: *Candida*; candidiasis, oral; drug resistance, fungal; stomatitis, denture.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

C.	Fungo do gênero *Candida*
sp.	Uma espécie de um gênero
spp.	Várias espécies de um gênero
CDDB	Centro de Diagnóstico das Doenças da Boca
CEP	Comitê de Ética em Pesquisa
DNA	*Deoxyribonucleic Acid* (ácido desoxirribonucléico)
EPI	Equipamento de proteção individual
NCTBIO	Núcleo de Biologia Celular e Tecidual
TCLE	Termo de Consentimento Livre e Esclarecido
UFPEL	Universidade Federal de Pelotas
FO	Faculdade de Odontologia
CAC	Candidíase Atrófica Crônica

SUMÁRIO

1 Introdução e Revisão de Literatura

1.1 Candida spp.

Os fungos são frequentemente encontrados como componentes normais da microbiota bucal. Entre as espécies, a mais comum é a *Candida*, em especial a *C. albicans* (DANGI et al., 2010; FALAGAS et al., 2010). Em alguns casos, este fungo é o responsável por uma série de doenças oportunistas que afetam a boca, mas também o trato gastrointestinal e vaginal (RAO, 2012; SCHWINGEL et al., 2012; TELLAPRAGADA et al, 2014). A apresentação clínica da *Candida* pode ser bastante variada, o que acarreta em comportamento biológico diferenciado, podendo crescer como hifas ou leveduras, sendo a primeira a forma patogênica (MIMA et al., 2010; MARTINS et al., 2011; SKUPIEN et al., 2013).

Na boca, a candidíase acontece como uma infecção oportunista, manifestando-se clinicamente com características distintas, como descritas a seguir:

- Candidíase pseudomembranosa aguda: placa branca ou amarelada que se desprende com raspagem, deixando uma região eritematosa ou erosiva e/ou ulcerada, com sintoma de leve queimação e pode ocorrer em qualquer região da mucosa oral;
- Candidíase atrófica aguda: pode ser consequência da pseudomembranosa, ou seja, não apresenta placa, apenas áreas de eritema localizadas principalmente no palato e dorso de língua;
- Candidíase hiperplásica crônica: caracterizada por placas brancas que não se desprendem com raspagem, mais comuns em mucosa retrocomissural;
- Candidíase atrófica crônica: região eritematosa, podendo apresentar superfície granular e algumas petéquias hemorrágicas, assintomática ou apresentando queimação, halitose, e secura. Outros locais de frequente ocorrência desta forma clínica é a língua (glossite romboidal mediana), comissura labial (queilite angular), e

no palato, justamente na região em contato com a prótese (estomatite por dentadura).

Esta última, principalmente associada a próteses superiores totais ou parciais removíveis, uma vez que os fungos têm uma grande afinidade pelas superfícies acrílicas, às quais se aderem (BARBEAU et al, 2003; KRAMER et al., 2006; MIMA et al., 2011; COSTA et al., 2013; BERTOLINI et al., 2014; KHOZEIMEH et al., 2014).

1.2 Candidíase Atrófica Crônica (Estomatite por Dentadura)

A candidíase atrófica crônica (CAC) é a manifestação mais comum de infecção por *Candida*, acometendo grande parte dos usuários de próteses superiores removíveis (mais de 65%). (GONÇALVES et al., 2012; OKAMOTO et al., 2012; CHAMBO FILHO et al., 2014). Essa alteração é diagnosticada durante o exame bucal, observando-se inflamação e eritema nas superfícies mucosas recobertas pela prótese, sendo por isso chamada estomatite por dentadura (GENDREAU e LOEWY, 2011; CAPISTRANO et al., 2013). Alguns fatores estão fortemente relacionados à doença, tais como a má higiene da prótese e o hábito de dormir com a prótese, principalmente em pacientes imunocomprometidos; que fazem uso de antibioticoterapia por longos períodos, causando alteração da microbiota oral; em pessoas com baixo padrão de higiene, portadores de endocrinopatias, em extremos de idade, alcoólatras e fumantes (CASSONE e CAUDA, 2012; GONÇALVES et al., 2012; RAO, 2012; SCHWINGEL et al., 2012). O diagnóstico é feito com exame clínico, sendo recomendado que se faça em conjunto com métodos microbiológicos, como a técnica do esfregaço (LUND et al., 2009). Existem alguns métodos para identificação das espécies de *Candida,* tais como PCR, o ChroMagar (através das cores das colônias), e também métodos automatizados, mais modernos, que também permitem testar a suscetibilidade aos antifúngicos (ALAM et al, 2014; CASTELLOTE e SORIANO, 2013; DAEF et al, 2014; TELLAPRAGADA et al, 2014). *Para* permitir uma melhor análise acerca da estomatite protética, Newton (NEWTON, 1962), criou uma classificação baseada nos aspectos clínicos das lesões, dividindo-as em três grupos:

Hiperemia puntiforme (classe I) - É definida pela hiperemia dos ductos de glândulas salivares palatinas menores; o que confere aspecto eritematoso

puntiforme, podendo ocupar áreas dispersas ou pequenas áreas localizadas no palato duro. (Figura 1):

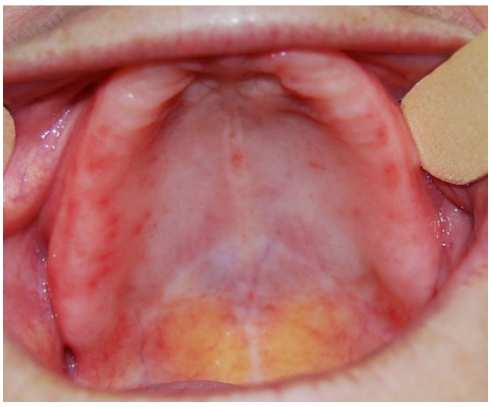

Figura 1 - CAC Classe I (imagem cedida pelo CDDB)

Hiperemia difusa (classe II) - É apontado por muitos estudos como o tipo mais comumente encontrado. Apresenta mucosa lisa e atrófica, com aspecto eritematoso em toda região sob a prótese. (Figura 2):

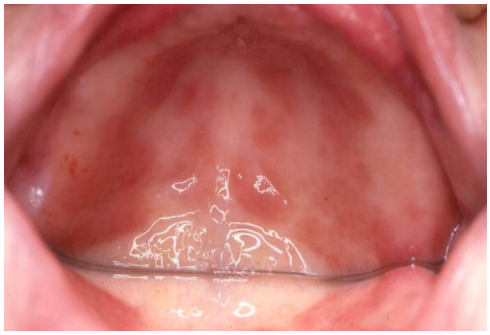

Figura 2 - CAC Classe II (imagem cedida pelo CDDB)

Hiperemia granular (classe III) - É frequentemente associada à câmara de sucção, acometendo a região central do palato com aparência clínica nodular e rugosa da mucosa. (Figura 3):

Figura 3 - CAC Classe III (imagem cedida pelo CDDB)

Todas as formas iniciam após o contato do fungo (*C. albicans*) com a mucosa do palato, e sua posterior invasão no tecido epitelial. (ALTARAWNEH et al., 2012).

Entre os fatores etiológicos da candidíase atrófica crônica estão: a interação entre a *C.albicans* e as células do hospedeiro, alterações do fluxo salivar, trauma de próteses desadaptadas, pobre higiene bucal e da prótese, além da possibilidade de resposta imune reduzida devido às condições sistêmicas. Apesar de o fungo se aderir à superfície da prótese, a substituição da mesma não seria a solução para curar a doença. (GENDREAU e LOEWY, 2011; SALERNO et al., 2011; ALTARAWNEH et al., 2012). Vale ressaltar que outros fatores também estão relacionados com essa patologia, tais como: produção de enzimas, adesão, dimorfismo, salivação, produção de toxinas, colonização bacteriana e, principalmente, aqueles ligados a defesa do hospedeiro (BUDTZ-JORGENSEN et al., 1988; PEREZOUS et al., 2006; KESIC et al., 2014).

1.3 Tratamentos antifúngicos e resistência antimicrobiana

O tratamento de escolha para a candidíase atrófica crônica é o uso de antifúngicos, geralmente na forma tópica, mas também o uso sistêmico pode ser necessário em alguns casos. Além disso, orienta-se o paciente a retirar a prótese para dormir, deixando-a mergulhada em solução com hipoclorito de sódio ou bicarbonato de sódio, e também a realizar higiene rigorosa das mesmas. (SALERNO et al., 2011; ANDRADE et al., 2013; ENAMI et al., 2014).

Entre os antifúngicos mais utilizados no tratamento desta lesão estão os Agentes Poliênicos (Nistatina); Agentes Azóis (Miconazol, Cetoconazol, Itraconazol e Fluconazol); e os Análogos do DNA (Fluorocitosina). Os Agentes Poliênicos modificam a permeabilidade da membrana citoplasmática do fungo a. A propriedade fungicida dos Agentes Azóis é a ligação à membrana dos fungos, destruindo os constituintes e a função da mesma. Os Análogos do DNA, por sua vez, interferem na síntese do ácido nucléico do fungo (SKUPIEN et al., 2013; LALLA e DONGARI-BAGTZOGLOU, 2014; TAY et al., 2014). Como tratamento sistêmico, por via endovenosa, a Anfotericina-B pode ser utilizada em casos extremos, por ser uma medicação extremamente forte e apresente efeitos colaterais imediatos como febre, calafrios e náuseas (DOVIGO et al., 2011; COSTA et al., 2012). O Fluconazol pode ser administrado por via oral ou endovenosa, mostrando-se uma alternativa viável,

especialmente para os casos de candidíase disseminada, devido à sua elevada eficácia e baixa toxicidade. O Miconazol também tem a apresentação comercial na forma de adesivos (KOSSIONI, 2011; SALERNO et al., 2011; AL-KHAFAJI, 2012).

O uso desses antifúngicos regride a estomatite por dentadura, na maior parte dos casos. No entanto, em algumas situações, a remissão da lesão é dificultada. Os maiores problemas encontrados são, a resistência ao antifúngico, a dependência de adesão ao tratamento por parte do paciente, muitas vezes dificultada pela idade do paciente, e também a ação de dissolução e limpeza da cavidade oral pela saliva (LALLA e DONGARI-BAGTZOGLOU, 2014). Todos estes fatores contribuem para reduzir a concentração dos antifúngicos tópicos, tornando o tratamento prolongado e as taxas de recorrência altas. (SALERNO et al., 2011; MIMA et al., 2012; MACIĄG et al., 2014). Essa exposição a concentrações subterapêuticas seriam um dos fatores responsáveis pelo aumento da patogenicidade de espécies de *Candida spp*, (COPPING et al., 2005; SANITÁ et al., 2013). Existe também a preocupação do aumento da patogenicidade de espécies menos virulentas que a *Candida albicans*, mas que são resistentes ao Fluconazol, como por exemplo, a *Candida krusei* (MESSER et al., 2006; COSTA et al., 2011; CAPISTRANO et al., 2013).

Enfim, o uso desses antifúngicos de forma indiscriminada favorece a seleção de cepas com reduzida sensibilidade ou até mesmo a manifestação do fenótipo resistente. Por sua vez, o perfil de sensibilidade de *Candida* spp aos antifúngicos difere, o que torna fundamental a identificação do agente etiológico causador da candidíase antes de iniciar a terapêutica empírica (ROZKIEWICZ et al., 2005; TAY et al., 2014). Ainda é importante considerar que a administração sistêmica de antifúngicos, apesar de efetiva, não elimina os microrganismos que colonizaram a superfície da prótese (SHAPIRO et al., 2011; JAVED et al., 2014).

2 Projeto de Pesquisa

A candidíase bucal é uma manifestação comum, de caráter crônico, que atinge grande parte dos indivíduos, principalmente os usuários de próteses dentárias. O tratamento tradicional com antifúngicos muitas vezes é prolongado e recorrente, colaborando para a manifestação de cepas. Portanto, é importante a realização de um estudo retrospectivo a fim de caracterizar o perfil demográfico e clínico dos pacientes portadores de candidíase bucal, bem como o isolamento, identificação e teste de suscetibilidade aos antifúngicos dos pacientes com candidíase atrófica crônica (MIMA et al., 2010; MARTINS et al., 2011; SKUPIEN et al., 2013; BERTOLINI et al., 2014; KHOZEIMEH et al., 2014).

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Entre os antifúngicos mais utilizados no tratamento desta lesão estão os Agentes Poliênicos (Nistatina, Anfotericina-B); Agentes Azóis (Miconazol, Cetoconazol, Itraconazol e Fluconazol); e os Análogos do DNA (Fluorocitosina) (DANGI et al., 2010; FALAGAS et al., 2010). Os Agentes Poliênicos modificam a permeabilidade da membrana citoplasmática do fungo a. A propriedade fungicida dos Agentes Azóis é a ligação à membrana dos fungos, destruindo os constituintes e a função da mesma. Os Análogos do DNA, por sua vez, interferem na síntese do ácido nucléico do fungo (SKUPIEN et al., 2013; LALLA e DONGARI-BAGTZOGLOU, 2014; TAY et al., 2014). Como tratamento sistêmico, por via endovenosa, a Anfotericina-B pode ser utilizada, embora apresente efeitos colaterais imediatos como febre, calafrios e náuseas (DOVIGO et al., 2011; COSTA et al., 2012; COSTA et al, 2013). O Fluconazol pode ser administrado por via oral ou endovenosa, mostrando-se uma alternativa viável, especialmente para os casos de candidíase disseminada, devido à sua elevada eficácia e baixa toxicidade. O Miconazol também tem a apresentação comercial na forma de adesivos (KOSSIONI, 2011; SALERNO et al., 2011; AL-KHAFAJI, 2012).

Este trabalho teve como objetivo realizar um estudo retrospectivo dos casos de candidíase bucal de um Centro de Diagnóstico Bucal do Sul do Brasil, a fim de caracterizar o perfil demográfico e clínico dos pacientes com diagnóstico de candidíase oral. Além disso também foi realizado o isolamento, identificação fúngica e teste de suscetibilidade antifúngica de amostras de *Candida*, coletadas de pacientes diagnosticados com candidíase atrófica crônica, a fim de determinar o melhor tratamento de pacientes com resistência antifúngica.

2.1 Objetivos

2.1.1 Geral

Realizar um estudo retrospectivo dos casos de candidíase bucal de um Centro de Diagnóstico Bucal do Sul do Brasil e identificação de leveduras isoladas e sua suscetibilidade em pacientes com candidíase atrófica crônica.

2.1.2 Específicos

Caracterizar o perfil demográfico e clínico dos pacientes com diagnóstico de candidíase oral;
Isolar e identificar amostras de *Candida* em pacientes diagnosticados com candidíase atrófica crônica.
Determinar o melhor tratamento de pacientes com resistência antifúngica através do método automatizado Vitek 2 (Biomeriéux).

2.2 Metodologia

2.2.1 Estudo retrospectivo

Primeiramente, serão analisados todos os livros de registro de atendimento clínico do Centro de Diagnóstico de Doenças da Boca (CDDB) da Faculdade de Odontologia da Universidade Federal de Pelotas. Nesses livros serão selecionados os pacientes que tenham sido diagnosticados com alguma forma clínica de candidíase, entre elas citam-se: glossite romboidal mediana, queilite angular, candidíase atrófica crônica, candidíase hiperplásica e candidíase pseudomembranosa e/ou eritematosa aguda. Após essa primeira seleção, a coleta de dados dos pacientes será realizada nas fichas de registro dos pacientes (prontuários), sendo descartadas as que não estiverem claras ou devidamente preenchidas. Dados como raça, gênero, idade, doenças sistêmicas, tipo e localização da candidíase, sintomas e hábitos nocivos serão extraídos das fichas clínicas e tabulados em planilha confeccionada em programa Excel (Office 2010). (ANEXO C)

2.2.2 Seleção dos pacientes com Candidíase Atrófica Crônica

Serão selecionados, através de um levantamento nos livros de registro de atendimento clínico e nas fichas clínicas, pacientes provenientes do Centro de Diagnóstico de Doenças da Boca (Faculdade de Odontologia de Pelotas – Universidade Federal de Pelotas) que possuírem o diagnóstico clínico de Candidíase Atrófica Crônica. Serão incluídos tanto os que já foram submetidos à terapia antifúngica sem obtenção da remissão dos sinais e sintomas, e também aqueles

atendidos a partir do início da pesquisa, sem tratamento em curso. O período da pesquisa será de Abril (2014) à Março (2015). Estes pacientes serão informados sobre as etapas do estudo e serão convidados a participarem do mesmo. Os voluntários que aceitarem, assinarão o termo de consentimento livre e esclarecido (ANEXO A). Os voluntários selecionados para o estudo passarão por um novo exame clínico para confirmar o diagnóstico da candidíase bucal, encontrado nos prontuários do serviço. Ademais, serão questionados sobre o uso de medicamentos sistêmicos, doenças crônica e ou imunossupressoras, consumo de álcool e tabagismo (ANEXO B). Este projeto foi aprovado pelo Comitê de Ética em Pesquisada UFPel, sob parecer/documento número 033/2006 (ANEXO D).

O exame bucal será realizado usando um espelho bucal, espátula de madeira e compressas de gaze, bem como sob campo iluminado. O diagnóstico de candidíase atrófica crônica será estabelecido de acordo com os critérios de Newton (1962).

2.2.3 Isolamento, identificação das espécies de *Candida* e determinação do perfil de suscetibilidade aos agentes antifúngicos

Os pacientes selecionados serão submetidos à coleta de material microbiológico da mucosa bucal por meio de swabs estéreis e secos, os quais serão suavemente friccionados na mucosa afetada pela lesão (Lund et al., 2010).

Após a coleta o material, este será cultivado em placa de Petri contendo meio de cultura CHROMagar *Candida* (Probac do Brasil, São Paulo, SP, Brazil) e incubado a 30ºC por 48 horas. Após esse período e a constatação do crescimento de colônias sugestivas de *Candida spp*. as mesmas serão congeladas para posterior identificação. Estas serão identificadas através do Vitek 2 Compact (Biomérieux, Brasil), que emprega o cartão “YST Test Kit” específico para identificação fúngica. O VITEK 2 é integrado com a aplicação do software Myla, que centraliza informações em um painel de comandos principal, para acompanhar os testes de preparação e análise das amostras. Após a cultura, os isolados são simultaneamente introduzidos no VITEK 2, páginas eletrônicas são geradas automaticamente, garantindo a rastreabilidade completa da amostra do início ao fim. A partir daí, é fornecida a identificação do organismo através de provas bioquímicas.

O teste de resistência antifúngica será realizado com todas as amostras de *Candida* spp. coletadas dos pacientes. Esse teste será realizado com o cartão "AST YST 07 Test Kit" do Vitek2Compact (Biomérieux, Brasil), específico para antifungiograma, que testa os antifúngicos Fluocitosina, Fluconazol, Voriconazol, Anfotericina b, Caspofungina, Micafungina.

2.3 Análise estatística

2.3.1 Estudo retrospectivo

A análise estatística será realizada através do software STATA 13.1. Para avaliação dos fatores de risco para candidíase atrófica crônica (CAC) será usado a Regressão de Poisson com variância robusta ($p \leq 0,05$).

2.3.2 Isolamento, identificação, suscetibilidade antifúngica e prevalência de variáveis associadas com candidíase atrófica crônica.

A análise estatística será realizada utilizando o STATA 13.1. O teste estatístico utilizado será o Qui-Quadrado, com nível de significância de 5%.

2.4. Resultados Esperados

Com este trabalho será possível caracterizar o perfil demográfico e clínico dos pacientes que procuram o serviço do Centro de Diagnostico de Doenças da Boca, podendo-se avaliar tempo de tratamento, medicação administrada, informações sobre a saúde dos pacientes, higiene e sobre a prótese.

Também será possível identificar as cepas de *Candida* spp. resistentes criando-se uma alternativa mais eficaz e específica para pacientes que estão em tratamento com antifúngicos usuais sem obter remissão dos sinais clínicos e cura.

Os resultados desta pesquisa serão divulgados em Congressos e os resultados finais serão publicados em periódicos científicos de relevância internacional.

2.5 Cronograma

	2014										2015						
Atividades	03	04	05	06	07	08	09	10	11	12	01	02	03	04	05	06	07
Revisão da Bibliografia	x	x															
Elaboração do projeto	x	x															
Seleção dos pacientes	x	x	x	x	x	x	x	x	x	x	x	x	x				
Isolamento e identificação dos microrganis-mos				x	x	x	x	x	x	x	x	x	x	x	x		
Organização dos Dados e Divulgação dos resultados														x	x		
Redação do artigo científico														x	x	x	
Submissão em periódico																	x
Defesa da Dissertação																	x

2.6 Orçamento

Item	Quantidade	Valor unitário	Valor total
Eppendorff	1	R$ 100,00	R$ 100,00
VITEK2 TUBOS PLASTICO 12X75MM 2.000UND (Biomerieux)	1	R$ 598,40	R$ 598,40
VITEK2 SALINA 0,45% - 1.000ML (Biomerieux)	4	R$ 72,60	R$ 290,40
VITEK YST TEST KIT (Biomerieux)	4	R$ 376,64	R$ 1.506,56
VITEK AST YS07 (Biomerieux)	4	R$ 376,64	R$ 1.506,56
CHROMagar (Probac) 500g	2	R$ 257,00	R$ 514,00
Folhas de papel (cópias)	1 20	R$ 0,20	R$ 24,00
TOTAL			**R$ 4.539,92**

3 Relatório de Trabalho de Campo

Os projetos iniciaram seus desenvolvimentos no mês de abril de 2014 pelo Programa de Pós Graduação em Odontologia, área de concentração Diagnóstico Bucal. A realização do trabalho "**Estudo retrospectivo de 1534 casos de candidíase oral no Sul do Brasil: levantamento de 18 anos"** se deu no Centro de Diagnóstico de Doenças da Boca (CDDB) da Faculdade de Odontologia, da UFPel, sendo realizado por um aluno de mestrado e uma aluna de graduação, ambos da Faculdade de Odontologia da Universidade Federal de Pelotas. O trabalho "**Isolamento, identificação, suscetibilidade antifúngica e prevalência de variáveis associadas com candidíase atrófica crônica"** se deu nas clínicas de atendimento da disciplina de UDE II e do Centro de Especialidades Odontológicas (CEO) de Diagnóstico Bucal, além do Laboratório de Microbiologia Oral da Faculdade de Odontologia da UFPel. Este trabalho foi realizado por um aluno de mestrado da Faculdade Odontologia da Universidade Federal de Pelotas e por dois professores (Faculdades de Odontologia e de Medicina Veterinária). Desta forma, o mestrando pôde atuar de maneira interdisciplinar, permitindo uma ampla atuação e participação do mesmo em diversas linhas de pesquisa.

Durante a realização das disciplinas obrigatórias para obtenção do título requerido, no primeiro ano foi realizada a revisão bibliográfica a respeito do tema proposto, elaboração do projeto, e início da execução do trabalho, através da avaliação das fichas clínicas dos pacientes com diagnóstico clínico de candidíase oral (artigo 1), e isolamento das espécies de fungo dos pacientes com diagnóstico clínico de candidíase atrófica crônica (artigo 2).

No segundo ano foi realizada a tabulação dos dados pertinentes ao artigo 1, além da identificação fúngica e teste de resistência aos antifúngicos pelo sistema automatizado Vitek 2, para finalização do artigo 2. Após, foi realizada a organização dos resultados, análise estatística e redação dos artigos.

O objetivo do primeiro trabalho (Estudo retrospectivo de 1534 casos de candidíase oral no Sul do Brasil: levantamento de 18 anos) foi de caracterizar o perfil demográfico e clínico dos pacientes com diagnóstico de candidíase oral, enquanto do segundo trabalho (Isolamento, identificação, suscetibilidade antifúngica e prevalência de variáveis associadas com candidíase atrófica crônica) foi de isolar e identificar amostras de *Candida* em pacientes diagnosticados com candidíase atrófica crônica, e determinar o melhor tratamento de pacientes com resistência antifúngica através do método automatizado Vitek 2 (Biomeriéux). Em ambos os trabalhos, foi possível alcançar os objetivos propostos, permitindo assim a redação de dois artigos científicos de grande valia para o meio acadêmico e de pesquisa.

4 Artigo 1

Oral candidiasis in Southern Brazil: an 18-year retrospective survey of 1,534 cases

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ABSTRACT

Objectives: To conduct a survey of the demographic and clinical profile of patients diagnosed with candidiasis and treated at the Center for Diagnosis of Oral Diseases (Centro de Diagnóstico de Doenças da Boca, CDDB), Universidade Federal de Pelotas School of Dentistry (FO/UFPel), from 1997 to 2014. **Materials and Methods:** Using a retrospective, cross-sectional, epidemiological design, data on variables such as race, gender, age, systemic diseases, type and location of oral candidiasis, symptoms, and harmful habits were collected. Statistical analysis was performed using STATA version 13.1. Assessment of risk factors for chronic atrophic candidiasis (CAC) was performed using Poisson regression with robust variance ($p \leq 0.05$). **Results:** The majority of patients seen at CDDB over the 18-year period of analysis (1997–2014) with a clinical diagnosis of oral candidiasis were white women, aged 51–60 years, nonsmokers and nondrinkers, with no systemic diseases, and who wore some form of dental prosthesis. The most prevalent type of candidiasis was CAC, the most commonly affected site was the palate, and the most widely used treatment was the standard protocol of the study facility, whereby patients are able to choose among nystatin cream, Micostatin®, or Daktarin® gel. Most patients were asymptomatic and had no comorbid oral lesions. According to clinical records, the majority of patients had a treatment duration of <1 year. **Conclusions:** Clinical Relevance: The regular publication of epidemiological data has an extremely important role to play in the implementation of prevention campaigns and in raising awareness of the etiologies of oral candidiasis.

Keywords: *Candida*; oral candidiasis; antifungal drug resistance; denture-related stomatitis.

INTRODUCTION

Oral candidiasis is the most common fungal infection of the mouth. In the majority of cases, it manifests as a chronic condition, with varying degrees of severity. Oral candidiasis affects a large portion of the population, particularly at the extremes of age (children and older adults), and is particularly frequent in those who wear dental prostheses. It is considered an opportunistic infection, occurring in persons with impaired immunity [1]. According to Dangi et al. [2], candidiasis does not manifest in healthy patients. For candidiasis to develop, the competitive ability of the host microbiota must be impaired, which facilitates growth of the fungus. However, the mere presence of the fungus is not indicative of manifest infection. This requires tissue penetration, which usually only occurs under select circumstances [3,4].

The most commonly implicated species is *Candida*, especially *C. albicans* [2,5]. This fungus is the causative agent of several opportunistic conditions that affect the mouth, gastrointestinal tract, and vagina [3]. Other, less common species may also be present, such as *C. parapsilosis, C. tropicalis, C. glabrata, C. krusei, C. pseudotropicalis, C. guilliermondi, C. stellatoidea*. The clinical presentation of *Candida* infection can vary widely, as the result of distinct biological behaviors; the fungus may grow as hyphae or as yeasts, the former being the pathogenic form [6,1].

In the oral cavity, candidiasis occurs as an opportunistic infection with distinct clinical characteristics in its acute and chronic forms, as follows:

- Acute pseudomembranous candidiasis: white or yellowish slough that can be scraped away, revealing erythematous, eroded and/or ulcerated mucosa; symptoms include mild burning sensation, and it can affect any region of the oral mucosa;
- Acute atrophic candidiasis: can be a consequence of pseudomembranous candidiasis. There is no slough, only areas of erythema, particularly on the palate and dorsum of the tongue;

- Chronic hyperplastic candidiasis: characterized by white plaques that do not slough or rub off, most commonly on the retro-commissural mucosa;
- Chronic atrophic candidiasis: characterized by areas of erythema, sometimes with a granular surface and scant petechial hemorrhages; it may be asymptomatic or associated with burning sensation, halitosis, and dry mouth. Other sites where this clinical form is common include the tongue (median rhomboid glossitis), commissure of the lips (angular cheilitis), and on areas of the palate that come into contact with a dental prosthesis (denture-related or denture-induced stomatitis). Denture-related stomatitis is mostly associated with full upper dentures and removable partial dentures, as fungi exhibit high a affinity to the acrylic surfaces of these prostheses, to which they adhere [7,8].

The treatment of choice for candidiasis is administration of antifungal agents, usually via the topical route, although systemic therapy may be required in some cases [9,10].

The antifungal agents most commonly used for treatment of this condition are the polyene antifungals (nystatin), the azole antifungals (miconazole, ketoconazole, itraconazole, and fluconazole), and the DNA analog flucytosine. Polyene antifungals alter the permeability of the fungal cytoplasmic membrane. Azole antifungals bind to the membrane and destroy its constituent components, rendering it non-functional. DNA analogs, in turn, interfere with fungal nucleic acid synthesis [4,11]. For systemic therapy, intravenous amphotericin B can be administred in extreme cases, to be an extremely strong medication, and is associated with immediate adverse effects including fever, chills, and nausea [12,13]. Fluconazole may be administered orally or intravenously. It is a viable alternative, especially in cases of disseminated candidiasis, due to its high efficacy and low toxicity. Miconazole is also available in patch form [14,9,15].

The prognosis is excellent. Use of any of these antifungals generally leads to resolution of candidiasis within approximately 1 month. However, in some situations,

remission is difficult to achieve. The major challenges observed are resistance to antifungal agents; dependence on patient adherence to treatment, which is often hindered by advanced age; and dissolution of bucally administered agents and cleansing of the oral cavity by saliva [11]. These factors contribute to reduced concentrations of topical antifungals, which often leads to protracted treatment and high recurrence rates [9,16,17]. Finally, indiscriminate use of antifungal agents predisposes to selection of fungal strains with low sensitivity, and even to manifestation of resistent phenotypes. The sensitivity profile of *Candida* spp. to antifungals varies; therefore, it is paramount that the exact causative agent of candidiasis be identified prior to institution of empiric therapy [18,19]. In addition, clinicians must take into account that systemic antifungal therapy, however effective, cannot eliminate any microorganisms that have colonized the surface of the dental prosthesis [20,21].

The present study sought to conduct a retrospective survey of cases of oral candidiasis seen at a specialist center for oral diagnosis in Southern Brazil and to characterize the demographic and clinical profile of patients with a diagnosis of this condition.

METHODS

A total of 1,594 patient records with a listed diagnosis of oral candidiasis between the years 1997 and 2014 (18 years) from the Center for Diagnosis of Oral Diseases (Centro de Diagnóstico de Doenças da Boca, CDDB) at the Universidade Federal de Pelotas School of Dentistry (FO/UFPel) were reviewed. Of these, 60 record were excluded due to incompleteness or missing data. The data collection stage was carried out by two FO/UFPel students: a master's degree candidate and an undergraduate dental student. All 1,534 records included in the sample were assessed. The following data were collected and recorded: race, gender, age, systemic diseases, clinical form of candidiasis, type and location of candidiasis, symptoms, and harmful habits, such as smoking and alcohol abuse. The clinical forms of

candidiasis were classified as follows: median rhomboid glossitis, angular cheilitis, chronic atrophic candidiasis, hyperplastic candidiasis, and acute pseudomembranous and/or erythematous candidiasis.

Statistical analysis

Data were extracted from clinical records and tabulated in a Microsoft Office Excel 2010 spreadsheet, organized as follows: sex (male or female); age (<50 years, 51–60 years, 61–70 years, or >70 years); skin color (white or nonwhite); smoking (yes or no); alcohol intake (yes or no); presence of systemic diseases (yes or no); denture wear (yes or no); type of candidiasis (chronic atrophic candidiasis [CAC] or other); affected site (palate or other); and medication used (local protocol, nystatin cream, Daktarin gel, or other. The "local protocol" consists of giving the patient the choice of nystatin cream, Micostatin®, or Daktarin® gel); chief complaint (asymptomatic, pain, burning sensation, or other); comorbid oral lesions (yes or no); and treatment duration (<1 year, 1–2 years, or ≥3 years).

Qualitative variables were expressed as absolute and relative frequencies, and quantitative variables as mean (standard deviation). Data were tabulated and analyzed in STATA 13.1. Assessment of risk factors for CAC was performed using Poisson regression with robust variance at the 5% significance level.

RESULTS

The records of 1,534 patients with a clinical diagnosis of oral candidiasis, seen between 1997 and 2014 at the FO/UFPel Center for Diagnosis of Oral Diseases, were reviewed.

Most of these patients were women (n=1,232, 80.3%); men accounted for only 19.7% of the sample (n=302).

Regarding age, 415 (27.1%) were aged 50 years or younger, 446 (29.1%) were aged 51 to 60 years, 358 (23.3%) were 61 to 70 years old, and 315 (20.5%) were >70 years old.

Overall, 1,308 patients (86.1%) were white and 226 (13.9%) were nonwhite.

Regarding smoking, 1,089 patients (71.0%) self-reported as nonsmokers, while 445 (29.0%) were smokers.

Alcohol intake was reported by only 23 patients (1.5%); 1,511 (98.5%) did not report alcohol consumption.

Systemic diseases were absent in 937 patients (61.1%) and present in 597 (38.9%). Among the diseases present were high blood pressure, diabetes and depression.

Overall, 910 patients (59.3%) were denture wearers, while 624 (40.7%) were not (Table 1).

CAC was the most common type of candidiasis, diagnosed in 1,500 patients (95%). The other types accounted for 34 patients (5%) overall: 2% with median rhomboid glossitis, 2% with hyperplastic candidiasis, and 1% with pseudomembranous candidiasis (Table 2).

The most commonly affected site was the palate, which accounted for 1,388 (90.9%) of all cases; 146 (9.1%) occurred elsewhere in the oral cavity.

Regarding medication use, the protocol of service local for candidiasis was prescribed to 800 patients (52.2%). The “protocol” consists of giving the patient the choice of nystatin cream, Micostatin®, or Daktarin® gel. Nystatin cream was specifically prescribed to 467 patients (30.4%), and Daktarin® gel to 144 (9.4%). Other treatment types were administered to 123 patients (8%).

Most patients (n=937, 61.1%) were asymptomatic; 298 (19.4%) reported a burning sensation, 183 (11.9%) reported pain, and 116 (7.6%) reported other symptoms.

Regarding comorbidity with other oral lesions, 1,090 patients (71.1%) had no lesions other than candidiasis, whereas 444 (28.9%) had additional oral lesions.

The most prevalent treatment duration was <1 year, observed in 1,003 patients (70.3%); 304 (17.7%) were on treatment for 1–2 years, and 227 (12%) had a treatment duration of >2 years (Table 2).

Prevalence analysis of risk factors for CAC yielded the following results.

Overall, 1,500 patients were diagnosed with CAC: 1,204 women (95.7% of the women in the sample) and 296 men (91.1% of the men in the sample). This difference was statistically significant.

Regarding age, 406 (91.1%) of patients aged <50 years, 436 (96.0%) of those aged 51–60 years, 350 (95.3%) of those aged 61–70 years, and 308 (97.5%) of those aged >70 years had CAC. Again, these differences were significant.

Of the 1,500 patients with CAC, 1,262 were white, and 238 were nonwhite; 1,064 were nonsmokers, while 436 smoked; 1,477 did not report alcoholism, whereas 23 did; 937 had no systemic diseases, whereas 563 had some systemic condition; 610 did not wear any type of dental prosthesis, while 890 were denture wearers. These analyses yielded no statistical differences (Table 3).

DISCUSSION

This epidemiological study was carried out in a region of Southern Brazil with a population of approximately 350,000, distributed across urban and rural areas (Source: IBGE). The FO/UFPel Center for Diagnosis of Oral Diseases (CDDB) is a referral center located in the city of Pelotas, state of Rio Grande do Sul, Southern Brazil, which operates within the Unified Health System (Sistema Único de Saúde, SUS) framework. On average, the center sees 250 patients per month, both from within Pelotas and from neighboring municipalities (Rio Grande, Piratini, Canguçu, Morro Redondo, and others).

Studies suggest that women are affected by oral candidiasis more often than men; however, it is known that women are more likely to seek medical attention and that incidence increases with advancing age, i.e., older adults have a higher prevalence of this condition, particularly due to difficulties in oral hygiene and to denture use [22-25]. In the present study, women were affected four times as much as men, and there was no significant difference in presence of the condition across age ranges.

Candidiasis is often associated with local conditions, such as other oral lesions (traumatic fibromas, aphthous stomatitis, carcinoma, etc.), or systemic diseases, such as diabetes, cardiovascular disorders, depression, and immunosuppression. However, the literature is not clear on the direct relationship between the presence of systemic diseases and oral candidiasis. Smokers are also strong "candidates" [26,27]. The present study found that most individuals afflicted with oral candidiasis were nonsmokers with no systemic diseases and no comorbid oral lesions.

Denture-associated angular cheilitis is found in patients with deep creases at the angle of the mouth, usually caused or aggravated by wearing full dentures with an incorrect vertical dimension, which creates points of low oxygenation at the labial commissures [13,28,11]. In our sample, fewer than 10% of patients had angular cheilitis; in most cases, it was associated with palate lesions. Other forms of candidiasis are less common than CAC, but no less clinically important. Pseudomembranous candidiasis can affect individuals of any age, but is especially common in debilitated patients and those living with chronic illness. This form of candidiasis presents as white or yellowish plaques or nodules that are easily rubbed off [16,17].

Hyperplastic candidiasis is a generally asymptomatic condition that presents as a hard lesion with a smooth, nodular, or fissured surface, ranging in color from white to red. This lesion is usually located on the dorsum of the tongue, in front of the vallate papillae, and

has a rhomboid border; hence, its alternate name of median rhomboid glossitis [26,19]. In the present study, corroborating the findings of Kramer et al. [29], Khozeimeh et al. [30], and Pedersen et al. [27], the palate was the site most commonly affected by candidiasis, which can occur on the dorsum of the tongue, the labial commissure, and the alveolar ridge, and the most common type of candidiasis was CAC.

The treatment of choice for *Candida albicans* infection involves pharmacotherapy with antifungals such as nystatin, administered as suspension, applied topically onto the lesion, or in the form of tablets and ointments. Ketoconazole is recommended particularly for chronic lesions and disseminated infection. Nystatin cream is used in cases of denture-related stomatitis, in which it is applied to the affected tissues as well as to the prosthesis, thus providing prolonged contact and eliminating any microorganisms present at the base of the denture. Nystatin oral suspension, when made to remain in contact with the oral lesion, can be used even for treatment of chronic and severe cases, with good clinical outcomes. Other medications, such as Daktarin® gel and Micostatin®, also provide high treatment efficiency. However, in most cases, treatment is slow and protracted, sometimes requiring months to induce remission, and potentially causing discomfort and discontinuation by patients. It can be said that the thrush has a high recurrence rate , so it reinforces the need to be set up the right treatment for this disease [19,31].

CONCLUSIONS

With this work , we managed to establish the clinic and demographic profile of patients with clinic diagóstico oral candidiasis. All healthcare providers, including dentists, dental hygienists and assistants, as well as public and private institutions and non-governmental organizations, should be involved in the promotion of preventive measures and in educating the entire population, with a particular emphasis on individuals who wear dental

prostheses. The regular publication of epidemiological data has an extremely important role to play in the implementation of prevention campaigns and in raising awareness of the etiologies of oral candidiasis.

ETHICAL STANDARDS

This study was approved by the Research Ethics Committee of Universidade Federal de Pelotas (UFPel), Pelotas, Rio Grande do Sul, Brazil. For this type of study formal consent is not required.

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TABLES

Table 1. Profile of 1,534 patients seen at an Oral Diagnosis Center in Southern Brazil,

Variable	n	%	95%CI	
Sex				
Female	1,232	80.3	78.2	82.2
Male	302	19.7	17.8	21.8
Age (years)				
<50	415	27.1	24.9	29.3
51–60	446	29.1	26.9	31.4
61–70	358	23.3	21.3	25.5
70 or older	315	20.5	18.6	22.6
Skin color				
White	1,308	86.1	84.2	87.7
Non-white	226	13.9	12.3	15.8
Smoking				
No	1,089	71.0	68.7	73.2
Yes	445	29.0	26.8	31.3
Alcohol consumption				
No	1,511	98.5	97.8	99.0
Yes	23	1.5	1.0	2.2
Systemic conditions				
Absent	937	61.1	58.6	63.5
Present	597	38.9	36.5	41.4
Denture wearer				
No	624	40.7	38.2	43.2
Yes	910	59.3	56.8	61.8

Table 2. Prevalence of oral candidiasis stratified by type and treatment characteristics in 1,532 patients seen at an Oral Diagnosis Center in Southern Brazil.

Variable	**n**	**%**	**95%CI**	
Type of candidiasis				
CAC	1,500	95	93.6	95.8
Other	34	5	4.2	6.4
Site				
Palate	1,388	90.9	89.4	92.3
Other	146	9.1	7.7	10.6
Medication used				
Protocol	800	52.2	49.6	54.6
Nystatin cream	467	30.4	28.2	32.8
Daktarin	144	9.4	8.0	11.0
Other	123	8.0	6.8	9.5
Chief complaint				
Asymptomatic	937	61.1	58.6	63.5
Bodily pain	183	11.9	10.4	13.7
Burning	298	19.4	17.5	21.5
Other	116	7.6	6.3	9.0
Other oral lesions present				
No	1,090	71.1	68.7	73.3
Yes	444	28.9	26.7	31.3
Treatment duration				
< 1 year	1,003	70.3	67.8	72.7
1 to 2 years	304	17.7	15.7	19.8
≥3 years	227	12.0	10.3	13.8

CAC, chronic atrophic candidiasis.

Table 3. Risk factors for chronic atrophic candidiasis in 1,500 patients seen at an Oral Diagnosis Center in Southern Brazil.

Variable	n	%	95%CI		p-value*
Sex					0.009
Female	1,204	95.7	94.4	96.7	
Male	296	91.1	87.3	93.8	
Age (years)					0.002
<50	406	91.1	87.9	93.5	
51–60	436	96.0	93.7	97.4	
61–70	350	95.3	92.5	97.0	
≥70	308	97.5	95.0	98.7	
Skin color					0.48
White	1,262	95.3	94.0	96.3	
Non-white	238	91.4	86.7	94.5	
Smoking					0.49
No	1,064	95.0	93.6	96.2	
Yes	436	94.2	91.6	96.0	
Alcohol consumption					0.15
No	1,477	95.0	93.7	96.0	
Yes	23	82.6	61.2	93.5	
Systemic conditions					0.06
Absent	937	95.7	94.2	96.9	
Present	563	93.3	91.0	95.0	
Denture wearer					0.18
No	610	95.7	93.8	97.0	
Yes	890	94.2	92.5	95.5	

*Poisson regression analysis with robust variance for estimation of prevalence ratios (PRs)

5 Artigo 2

Isolation, identification, and antifungal susceptibility of causative agents of chronic atrophic candidiasis and prevalence of associated variables

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Este trabalho será submetido para “Mycoses”

ABSTRACT

Background: The treatment of choice for chronic atrophic candidiasis (CAC or denture-related stomatitis) is antifungal therapy, generally via the topical route. Prolonged, recurrent use of antifungals contributes to the development of resistant species.

Objectives: The present study sought to isolate, identify, and assess the antifungal susceptibility of oral yeasts in patients diagnosed with CAC and evaluate the presence of variables associated with this condition.

Patients/Methods: Samples were collected with sterile swabs and seeded in CHROMagar Candida culture medium, obtained from patients with a clinical and laboratory-confirmed diagnosis of denture-related stomatitis. Species were isolated, identified, and their susceptibility to antifungal agents assessed by means of the automated Vitek 2 method (BioMérieux). Statistical analysis was performed by means of the chi-square test, at the 5% significance level, using STATA version 13.1.

Results: Overall, 44 patients with a clinical diagnosis of CAC were examined. Yeasts were isolated from 38 patient samples. The most prevalent species was *Candida albicans*. In these patients, the most prevalent Newton classification was type II. None of the species identified was resistant to the antifungals tested.

Conclusions: Our findings suggest that antifungal susceptibility testing and proper fungal identification can help dentists determine the optimal course of treatment for CAC.

Keywords: *Candida*; yeasts; antifungal drug resistance; denture-related stomatitis; comparative study.

INTRODUCTION

Oral candidiasis is a common, chronic condition that affects a large portion of the population, especially denture users. Traditional antifungal therapy is often prolonged and recurrent, which contributes to the manifestation of resistant strains. Within this context, retrospective studies are necessary to characterize the demographic and clinical profile of patients with oral candidiasis, as well as to isolate, identify, and test the antifungal drug susceptibility of causative agents involved in chronic atrophic candidiasis (CAC) [1-5].

CAC is the most common manifestation of *Candida* infection, and affects more than 65% of individuals who wear maxillary partial dentures [6-8]. It is usually detected during intraoral examination as inflammation and erythema on mucous surfaces that are covered by the dental prosthesis, hence the name denture-related stomatitis [9, 10]. Some factors are strongly associated with CAC, such as poor denture hygiene and the habit of sleeping with one's denture in, particularly in immunocompromised patients; those on long-term antibiotic therapy, which causes changes in the oral microbiota; those with poor general hygiene practices; those with endocrine disorders conditions; at the extremes of age; and in alcoholics and smokers [6, 11-13]. The diagnosis is established by clinical examination, but adjunct use of microbiological methods, such as the oral smear technique, is advisable [14]. Several methods are available for identification of *Candida* species, such as the polymerase chain reaction (PCR), ChroMagar (based on colony color), and modern automated methods, which allow for simultaneous testing of susceptibility to antifungals [15-18]. To enhance analysis of denture-related stomatitis, Newton [19] created a classification that divides the condition into three groups or classes based on the clinical appearance of lesions:

Pinpoint hyperemia (class I): Defined by hyperemia of the ducts of minor palatal salivary glands, which confers an erythematous, pinpoint-rash appearance to the lesion; it may cover dispersed areas or small, localized areas of the hard palate.

Diffuse hyperemia (class II): Reported in many studies as the most commonly found type. Characterized by a hyperemic, smooth, atrophic mucosa over the entire denture area.

Granular hyperemia (class III): Frequently associated with the suction chamber of the denture, this form affects the central region of the palate, conferring a nodular, wrinkled appearance to the mucosa.

All forms are triggered by contact of the causative fungus (*C. albicans*) with the palate mucosa and subsequent invasion of epithelial tissue [20].

The etiological factors of CAC include the interaction between *C. albicans* and host cells, changes in salivary flow, trauma due to denture misfit, poor oral hygiene and denture cleaning practices, and, potentially, a reduced immune response due to systemic conditions. Although the fungus adheres to the surface of the denture, replacement of the prosthesis is not curative [9, 20, 21]. It bears stressing that other factors are associated with CAC as well, including enzyme production, adhesion properties, dimorphism, salivation, toxin production, bacterial colonization and, particularly, factors related to host defenses [22-24].

The treatment of choice for CAC is administration of antifungal agents, usually via the topical route, although systemic therapy may be required in some cases. Furthermore, patients should be advised to remove their dentures before bed, soak them in sodium hypochlorite or sodium bicarbonate solution, and clean them thoroughly and often [21, 25-28].

The antifungal agents most commonly used for treatment of this condition are the polyene antifungals (nystatin), the azole antifungals (miconazole, ketoconazole, itraconazole, and fluconazole), and the DNA analog flucytosine [29, 30]. Polyene antifungals alter the permeability of the fungal cytoplasmic membrane. Azole antifungals bind to the membrane and destroy its constituent components, rendering it non-functional. DNA analogs, in turn, interfere with fungal nucleic acid synthesis [3, 31, 32]. For systemic therapy, intravenous amphotericin B can be administered in extreme cases, to be an extremely strong medication,

and is associated with immediate adverse effects including fever, chills, and nausea [33-35]. Fluconazole may be administered orally or intravenously. It is a viable alternative, especially in cases of disseminated candidiasis, due to its high efficacy and low toxicity. Miconazole is also available in patch form [21,36,37].

The present study sought to isolate and identify *Candida* in samples obtained from patients diagnosed with CAC and determine the optimal treatment strategy for patients with antifungal resistance.

METHODS

The sample comprised patients from the Center for Diagnosis of Oral Diseases (CDOD) at the Universidade Federal de Pelotas School of Dentistry (FO/UFPel), state of Rio Grande do Sul, Brazil, from April 2014 to March 2015. The study was approved by the FO/UFPel Research Ethics Committee (033/2006). All patients who wore full or partial dentures, had a clinical diagnosis of CAC, and agreed to participate in the study were included. Individuals who met the inclusion criteria (denture wearers; treated at CDOD; able to understand and complete the study questionnaire; willing and able to accept the study protocol and provide informed consent; with intraoral examination findings of diffuse or focal, erythematous, micropapular lesions confined to the palatal mucosa and clinically consistent with CAC, as well as dorsum of tongue lesions) were invited to participate and signed an informed consent form. The exclusion criteria were no denture use, other oral conditions that prevented immediate mycological diagnosis, and absence of clinical manifestations consistent with those described in the inclusion criteria. Forty-four patients underwent initial examination. All patients selected for inclusion in the study ($N = 44$) had agreed to participate. Volunteers underwent clinical examination for clinical manifestations of CAC and then completed a standardized questionnaire. Briefly, volunteers were interviewed

directly and the following variables were assessed clinically: appearance of denture-related stomatitis (Newton classification of CAC); type of denture (full, partial, or both); duration of denture use (less than 1 year, 2-5 years, 6-10 years, or more than 10 years); whether the participant follows denture hygiene practices (yes or no); whether the participant sleeps with dentures in (yes or no); history of antifungal therapy (yes or no); current smoking (yes or no); site of stomatitis (palate, tongue, or both); and systemic disease (presence or absence). The intraoral examination was performed by a master's candidate (previously trained and calibrated by an oral pathologist), under an operatory light, using a mouth mirror, wooden tongue depressor, and gauze pads. Diagnosis of CAC was performed as described elsewhere.

The selected patients underwent collection of microbiological material from the oral mucosa with the aid of sterile dry swabs, which were gently rubbed onto the affected mucosa [14].

After collection, the material was grown in Petri dishes containing CHROMagar *Candida* culture medium (Probac do Brasil, São Paulo, SP, Brazil) and incubated at 30ºC for 48 hours. After this period had elapsed and growth of colonies consistent with *Candida spp*. was apparent, the material was frozen for later identification. Species were identified with a VITEK 2 Compact system (Biomérieux, Brazil), which uses the YST Test Kit specific card for fungal identification. The VITEK 2 system is integrated with the Myla software suite, which combines information on a central dashboard for monitoring of sample preparation and analysis. After culture, isolates were simultaneously placed in the VITEK 2 system. An electronic workflow is generated automatically, ensuring complete sample traceability from beginning to end. Identification of the organism is then performed through biochemical assays.

Antifungal drug resistance tests were performed on all *Candida* spp. samples collected from patients. These tests were performed using the specific Vitek2Compact AST YST 07

Test Kit card (Biomérieux, Brazil), which tests the antifungals flucytosine, fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin.

Statistical analysis

Data were tabulated and analyzed in STATA 13.1. The Pearson chi-squared test was used to evaluate associations between Newton classification and the other variables of interest at the 5% significance level ($P \leq 0.005$).

RESULTS

Forty-four patients with a clinical diagnosis of CAC were examined. Samples from 38 patients were isolated, whereas samples from the remaining six did not exhibit growth after 48 hours of incubation on culture medium. Of the isolated samples, 34 grew only one species (33 *Candida albicans* and one *Candida guilliermondii*), whereas four grew two species (two *Candida albicans* + *Candida dubliniensis* and one case each of *Candida albicans* + *Candida glabrata* and *Candida albicans* + *Candida parapsilosis*) (Figure 1 and 2).

Associations between each of the isolated species and Newton classifications were also assessed. Thus, of the 38 isolates, six corresponded to Class I, 29 to Class II, and three to Class III cases (Figure 3).

Regarding antifungal susceptibility testing, none of the isolated species was resistant to the tested antifungals (Table 1).

Concerning the Newton classification of CAC, eight patients (18.2%) were in Class I, 33 (75%) in Class II, and three (6.8%) in Class III.

Regarding denture use, 28 patients (63.6%) wore full dentures, six (13.6%) wore removable partial dentures, and 10 (22.7%) wore both.

Only one patient (2.3%) had been wearing dentures for less than 1 year. Nine (20.5%) had been wearing dentures for 2 to 5 years, 20 (45.5%) for 6 to 10 years, and 14 (31.8%) for over 10 years.

Most patients (*N*=43, 97.7%) reported performance of denture hygiene practices. Only one (2.3%) claimed to not carry out any denture cleaning procedures.

When asked whether they slept with their dentures in place, 27 patients (61.4%) reported they did not, whereas the remaining 17 (38.6%) did.

Overall, 20 patients (45.4%) had received prior antifungal therapy, whereas 24 (54.6%) had not.

Ten patients (22.7%) were smokers and 34 (77.3%) were not.

The palate was the most frequently affected site, accounting for 43 cases (97.7%); only one patient (2.3%) had involvement of other sites (upper alveolar ridge).

Regarding presence of systemic diseases, 15 patients (34.1%) had some systemic condition, whereas 29 (65.9%) did not (Table 2). Systemic diseases reported by patients were hypertension, diabetes and depression. All patients reported being compensated with the disease through medication.

Table 3 shows associations between Newton classification and the other variables of interest to demonstrate whether any associations were actually found, i.e., the Newton classification of CAC of each case was cross-referenced with the type of denture, the duration of use, denture hygiene and sleeping practices, prior history of antifungal therapy for candidiasis, current smoking, site of candidiasis, and presence or absence of systemic disease. None of the associations were statistically significant.

DISCUSSION

Among the several clinical forms of candidiasis, CAC is considered one of the most ubiquitous of all opportunistic infections, frequently co-occurring with angular cheilitis, at the labial commissures, or in the "kissing lesion" of the dorsum of the tongue [8, 12, 13, 18].

Studies suggest that women are affected more often than men; however, it is known that women are more likely to seek medical attention and that incidence increases with advancing age, i.e., older adults have a higher prevalence of this condition, particularly due to difficulties in oral hygiene and to denture use [6, 12, 13]. This corroborates the findings of the present study, in which women were the majority of patients and represented the oldest group of denture wearers.

Candidiasis is often associated with local conditions, such as other oral lesions (traumatic fibromas, aphthous stomatitis, carcinoma, etc.), or systemic diseases, such as diabetes, cardiovascular disorders, depression, and immunosuppression [4, 8]. In the present study, the majority of patients had no systemic diseases and no other oral lesions.

Yeasts of the species *Candida albicans* account for 80-90% of cases of CAC, although other species can be implicated, such as *C. tropicalis, C. parapsilosis, C. glabrata, C. stellatoidea, C. guilliermondii,* and *C. krusei* [14,15, 35]. In the present study, essentially 90% of isolates were identified as *Candida albicans,* either as the sole species identified or as one of the species involved.

The most common Newton classification of denture-related stomatitis is Class II, which corresponds to an area of diffuse erythema on the palate [6, 19]; this was consistent with the present study. This distribution most likely occurs because the majority of patients with CAC wear complete dentures, which entails a larger surface area of contact between the prosthesis and palate. However, when the Newton classification was cross-referenced with the other variables of interest, there were no statistically significant differences.

Several methods are available for identification of *Candida* species, such as the polymerase chain reaction (PCR), ChroMagar (based on colony color), and modern automated methods, which allow for simultaneous testing of susceptibility to antifungals [16-18]. In this study, species were identified with the VITEK 2 Compact automated method (Biomérieux, Brazil), which uses the YST Test Kit specific card for fungal identification. The VITEK 2 system is integrated with the Myla software suite, which combines information on a central dashboard for monitoring of sample preparation and analysis. After culture, isolates are simultaneously placed in the VITEK 2 system. An electronic workflow is generated automatically, ensuring complete sample traceability from beginning to end. Identification of the organism is then performed through biochemical assays. This method also permits antifungal resistance testing of *Candida spp.* specimens collected from patients. These tests are performed using the specific Vitek2Compact AST YST 07 Test Kit card (Biomérieux, Brazil), which tests the antifungals flucytosine, fluconazole, voriconazole, amphotericin B, caspofungin, and micafungin [38, 39]. An alternative for better solution of cases of CAC CDOD is to introduce these antifungals tested on Vitek 2 , in routine service.

The treatment of choice for CAC is topical antifungal therapy, which leads to regression of denture-related stomatitis in the majority of cases. However, in some situations, remission is difficult to achieve. The major challenges observed are resistance to antifungal agents; dependence on patient adherence to treatment, which is often hindered by advanced age; and dissolution of bucally administered agents and cleansing of the oral cavity by saliva [31]. These factors contribute to reduced concentrations of topical antifungals, which often leads to protracted treatment and high recurrence rates [21, 40, 41]. All patients involved in this study were or are receiving topical antifungal therapy. In some cases, treatment is more prolonged than in others.

Exposure to subtherapeutic concentrations is believed to be one of the factors responsible for increased pathogenicity of *Candida* species [42, 43]. Potential increases in pathogenicity of species less virulent than *Candida albicans*, but resistant to fluconazole – such as *Candida krusei* – is also a concern [10, 44, 45]. In addition, clinicians must take into account that systemic antifungal therapy, however effective, cannot eliminate any microorganisms that have colonized the surface of the dental prosthesis [46-48]. One of the treatment strategies advocated is the "local protocol" of the facility where this study was conducted. This protocol consists of giving the patient a choice among nystatin cream, Micostatin, or Daktarin gel.

The efficacy of treatment depends on a variety of aspects, including patient cooperativeness, proper dosage, and proper treatment duration. Therefore, the optimal treatment strategy for CAC must be chosen on a case-by-case basis [8, 24]. All patients included in this study were or are receiving therapy and undergoing periodic follow-up.

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CONCLUSIONS

In the present study, the most prevalent Newton classification was type II, complete denture wearers were the most commonly affected group, and the palate was the predominant site of involvement. The most prevalent species isolated was *Candida albicans*. None of the species identified was resistant to the antifungals tested, which demonstrates that current indications for these agents are appropriate.

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TABLES

Table 1. Relationship between colonies isolated and resistance to the antifungals tested.

Fungus	n	%
C. albicans		
Resistant	0	0
Susceptible	37	100
C. dubliniensis		
Resistant	0	0
Susceptible	2	100
C. glabrata		
Resistant	0	0
Susceptible	1	100
C. parapsilosis		
Resistant	0	0
Susceptible	1	100
C. guilliermondii		
Resistant	0	0
Susceptible	1	100

Table 2. Sample profile.

Variable	*N*	%
Newton classification		
1	8	18.2
2	33	75.0
3	3	6.8
Type of denture		
Full	28	63.6
RPD	6	13.6
Both	10	22.7
Duration of denture use (years)		
1 or less	1	2.3
2 to 5	9	20.5
6 to 10	20	45.5
>10	14	31.8
Denture hygiene		
No	1	2.3
Yes	43	97.7
Sleeps with dentures in		
No	27	61.4
Yes	17	38.6
Prior antifungal therapy		
No	24	54.6
Yes	20	45.5
Smoking		
No	34	77.3
Yes	10	22.7
Site of stomatitis		
Palate	43	97.7
Tongue	0	0
Both	1	2.3
Systemic disease		
Present	15	34.1
Absent	29	65.9

Table 3. Associations between Newton classification and other variables (*N*=44)

Variable	Newton						*P*-value*
	1		2		3		
	n	%	n	%	n	%	
Type of denture							0.534
Total	5	17.9	22	78.6	1	3.6	
RPD	2	33.3	3	50.0	1	16.7	
Both	1	10.0	8	80.0	1	10.0	
Length of use							0.134
1 or less	1	100.0	0	0.0	0	0.0	
2 to 5	2	22.2	7	77.8	0	0.0	
6 to 10	5	25.0	14	70.0	1	5.0	
>10	0	0.0	12	85.7	2	14.3	
Daily hygiene							0.843
No	0	0.0	1	100.0	0	0.0	
Yes	8	18.6	32	74.4	3	7.0	
Sleeps with dentures in							0.206
No	6	22.2	18	66.7	3	11.1	
Yes	2	11.8	15	88.2	0	0.0	
Prior antifungal therapy							0.140
No	5	20.8	19	79.2	0	0.0	
Yes	3	15.0	14	70.0	3	15.0	
Smoking							0.879
No	6	17.7	26	76.5	2	5.9	
Yes	2	20.0	7	70.0	1	10.0	
Affected site							0.843
Palate	8	18.6	32	74.4	3	7.0	
Both	0	0.0	1	100.0	0	0.0	
Systemic disease							0.292
Present	4	26.7	11	73.3	0	0.0	
Absent	4	13.8	22	75.9	3	10.3	

*Pearson's chi-square test.

FIGURE LEGENDS

Figure 1. Flowchart of tests performed and species identified.

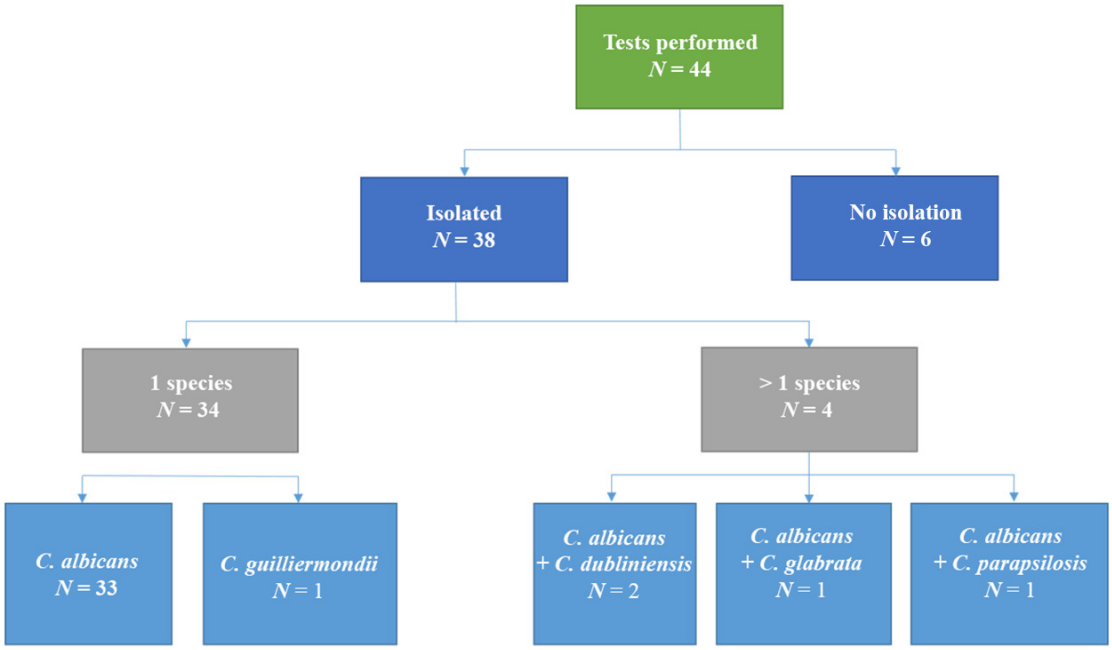


Figure 2. Distribution of patients by *Candida* species identified.

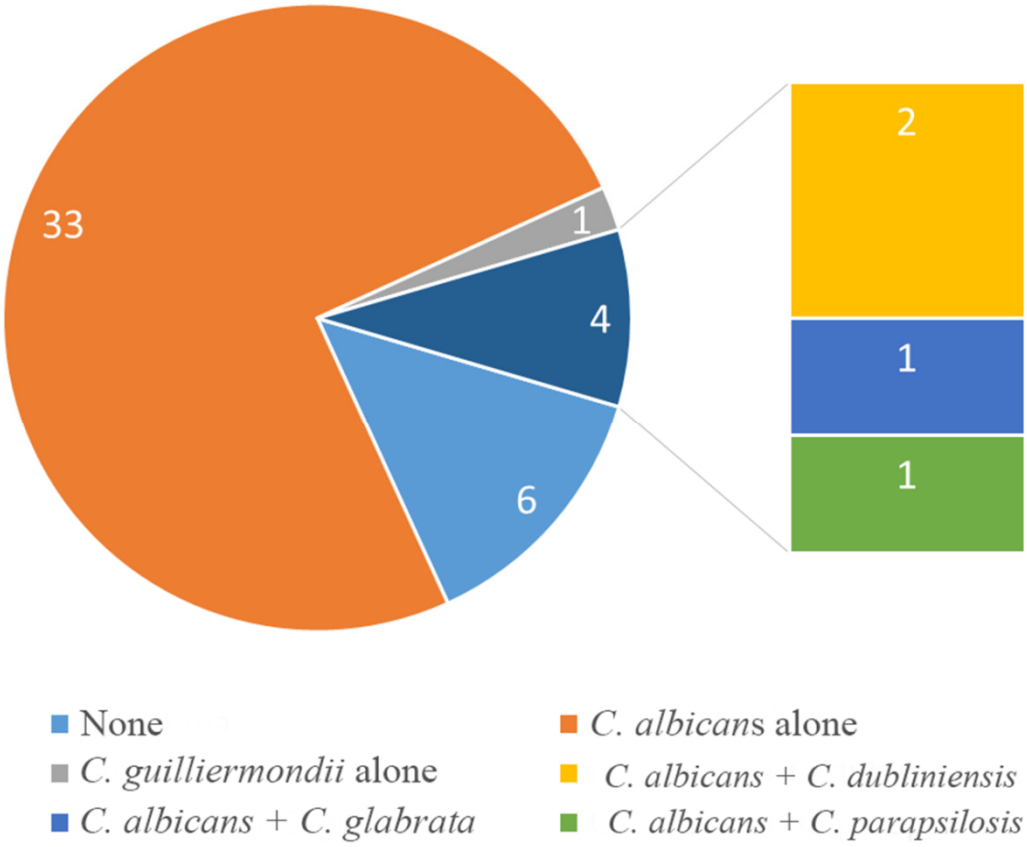
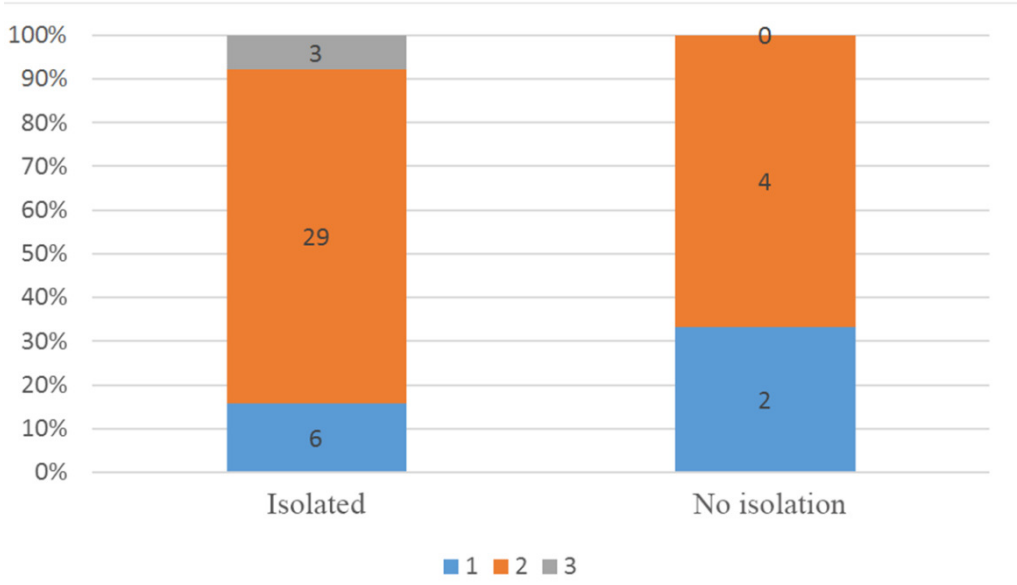


Figure 3. Association between isolation and Newton classification.



6. Considerações Finais

Com relação aos trabalhos realizados, pode-se dizer que o tipo de candidíase bucal mais prevalente é a candidíase atrófica crônica (CAC), o local mais acometido é o palato, a classificação de Newton mais prevalente é a do tipo II, e os usuários de prótese total são os mais acometidos. A espécie fúngica mais presente é a *Candida albicans*. A publicação regular de dados epidemiológicos é extremamente importante para a implementação de campanhas de prevenção e para uma maior consciência da etiologia das candidíases de origem bucal.

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ANEXOS

ANEXO A: Termo de Consentimento Livre e Esclarecido

Estudo retrospectivo de 1534 casos de candidíase oral no Sul do Brasil: levantamento de 18 anos e Isolamento, identificação, suscetibilidade antifúngica e prevalência de variáveis associadas com candidíase atrófica crônica.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Pesquisador Responsável: Leandro Calcagno Reinhardt – FO/UFPel
Telefones para contato: (53)91023104

Nome: _____
Idade: _____ anos R.G. _____

O Sr. (ª) está sendo convidado(a) a participar deste projeto de pesquisa. Este projeto tem como objetivos a identificação, quantificação e avaliação da resistência antifúngica de microrganismos coletados de pacientes que como o Sr(ª). Com isso poderemos delimitar o tipo dos microrganismos resistente bem como proporcionar para nossos pacientes uma proposta medicamentosa mais eficaz. Se o Sr(ª) aceitar participar desse estudo será submetido a uma entrevista inicial sobre o uso de medicamentos sistêmicos, bem como dados de doenças crônicas e ou imunossupressoras. Além disso, o Sr(ª) será perguntado sobre hábitos deletérios como por exemplo o uso do cigarro e álcool. O Sr(ª) será submetido a um novo exame clínico e um esfregaço da mucosa será realizado. Haverá a necessidade, em alguns casos, de repetições de coletas de saliva e em todos os casos o acompanhamento da regressão da doença. Como beneficio, poderemos ter uma analise mais detalhada do microrganismo que causa a sua patologia podendo sugerir uma opção de tratamento mais adequado. O Sr(ª) poderá obter informações sobre os testes e os resultados do projeto a qualquer momento com os responsáveis da pesquisa. É valido ressaltar que a participação do projeto é feita de forma voluntária e a não aceitação do mesmo não acarretará em prejuízo algum para o

voluntário. Este consentimento poderá ser retirado a qualquer momento, sem prejuízo ao voluntario. Garantimos a confidencialidade das informações dadas pelo Sr(ª) e a sua privacidade durante todas as etapas da pesquisa.

Eu, _____, RG nº _____ declaro ter sido informado e concordo em participar, como voluntário, do projeto de pesquisa acima descrito.

Pelotas, _____ de _____ de_____

_____

ANEXO B: Questionário de entrevista aos pacientes atendidos no Centro de Diagnóstico de Doenças da Boca

Ficha n°_____

Nome do paciente_____

Idade_____ Sexo_____

1)Usa prótese:

( ) total_____ ( ) parcial_____

2) ( ) mucossuportada ( ) dentossuportada ( ) dentomucossuportada

3) Quanto tempo usa prótese? ( ) até 1 ano ( ) até 5 anos ( ) até 10 anos ( ) + 10 anos

4) Costuma higienizar a prótese? ( ) Sim ( ) Não

5) Com que frequência? ( ) Semanalmente ( ) 1x ao dia ( ) +1x ao dia

6) Você dorme com a prótese? ( ) Sim ( ) Não

7) Apresenta alguma enfermidade? ( ) Sim ( ) Não

Qual(is)? _____

8) Você toma algum medicamento diário? ( ) Sim ( ) Não

9) Que tipo? ( ) antidepressivo ( ) anti-histamínico ( ) anti-inflamatório

( ) analgésico ( ) regulador de pressão ( ) regulador do açúcar

10) Faz ( ) ou fez ( ) algum tratamento antiviral, quimioterápico ou radioterápico?

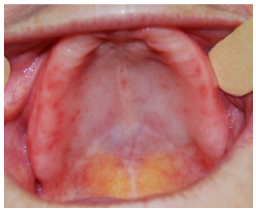
( ) Sim ( ) Não

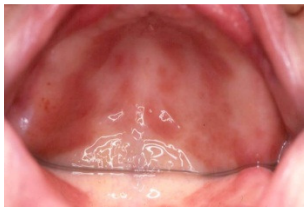
11) Fuma? ( ) Sim ( ) Não

12) Estomatite: ( ) palato ( ) palato e língua ( ) língua

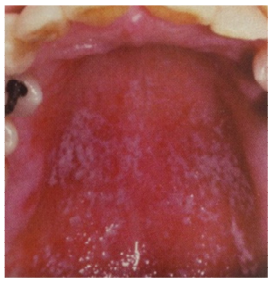
13) Classificação de Newton:

( ) classe I ( ) classe II ( ) classe III





14) ( ) lesão beijada



( ) glossite romboidal mediana

15) Fez ( ) ou faz ( ) uso de antifúngico? ( ) Sim ( ) Não

16) ( ) Tópico ( ) Sistêmico ( ) Ambos

16) Qual(is)_____

18) Há quanto tempo usa? ( ) -1 mês ( ) +1 mês ( ) até 6 meses ( ) até 1 ano ( ) +1 ano

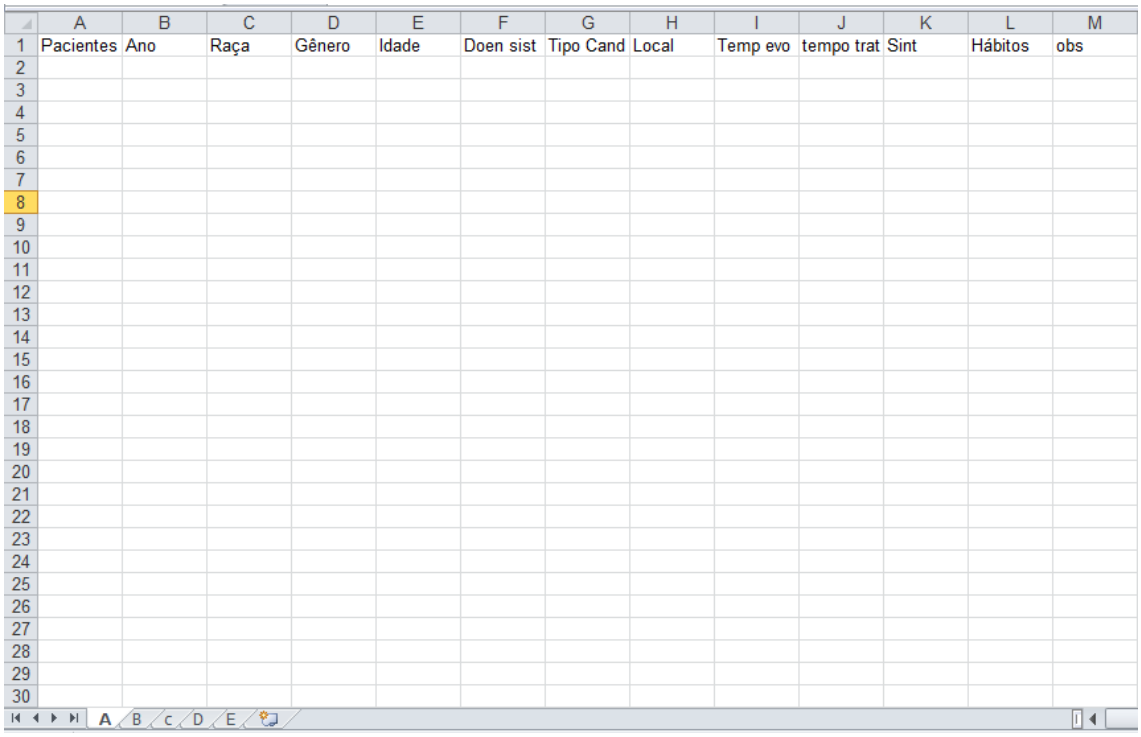
19) Com que frequência usa o antifúngico? ( ) -1x/dia ( ) +1x/dia

OBS.:_____

_____

_____

ANEXO C: Planilha com as informações dos prontuários dos pacientes do Estudo Retrospectivo



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Pacientes	Ano	Raça	Gênero	Idade	Doen sist	Tipo Cand	Local	Temp evo	tempo trat	Sint	Hábitos	obs
2													
3													
4													
5													
6													
7													
8													
9													
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11													
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A B C D E

ANEXO D: Parecer favorável do Comitê de Ética em Pesquisa sob o número 033/2006

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE ODONTOLOGIA
COMITÊ DE ÉTICA EM PESQUISA

PELOTAS, 09de janeiro de 2007

PARECER Nº 033/2006

O Projeto de pesquisa intitulado **"PREVALÊNCIA E GENOTIPAGEM DE ESPÉCIES DE CANDIDA RESISTENTES A ANTIFUNGICOS COLETADAS EM PACIENTES COM CANDIDÍASE ATRÓFICA CRÔNICA "** está constituído de forma adequada, cumprindo, na sua plenitudes preceitos éticos estabelecidos por este Comitê e pela legislação vigente, recebendo, portanto, **PARECER FAVORÁVEL** à sua execução. , o

Prof.Dr.Marcos Antônio Torriani
Coordenador do CEP/FO/UFEPel