Pedro Urquiza Jayme Silva

Uso associado de escovação dentária e clorexidina comparado ao uso exclusivo de clorexidina para prevenção de pneumonia associada à ventilação mecânica:

revisão sistemática e metanálise da literatura

Combination of tooth brushing and chlorhexidine compared to exclusive use of chlorhexidine to reduce the risk of ventilator-associated pneumonia:

a systematic review with meta-analysis

Dissertação apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia para obtenção de Título de Mestre em Clínica Odontológica.

Uberlândia, 2020

Pedro Urquiza Jayme Silva

Uso associado de escovação dentária e clorexidina comparado ao uso exclusivo de clorexidina para prevenção de pneumonia associada à ventilação mecânica:

revisão sistemática e metanálise da literatura

Combination of tooth brushing and chlorhexidine compared to exclusive use of chlorhexidine to reduce the risk of ventilator-associated pneumonia:

a systematic review with meta-analysis

Dissertação apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia para obtenção de Título de Mestre em Clínica Odontológica.

Orientador: Prof. Dr. Sérgio Vitorino Cardoso

Banca Examinadora:

Prof. Dr. Sérgio Vitorino Cardoso

Prof. Dr. Luiz Renato Paranhos

Prof. Dr. Luciano Alberto de Castro

Uberlândia, 2020

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Odontologia
Av. Pará, 1720, Bloco 4L, Anexo B, Sala 35 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: (34) 3225-8115/8108 - www.ppgoufu.com - copod@umuarama.ufu.br

ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Odontologia				
Defesa de:	Dissertação de Mestrado Acadêmico, número 368, PPGO				
Data:	Treze de fevereiro de dois mil e vinte	Hora de início:	09:00	Hora de encerramento:	10:45
Matrícula do Discente:	11812ODO012				
Nome do Discente:	Pedro Urquiza Jayme Silva				
Título do Trabalho:	Uso associado de escovação dentária e clorexidina comparado ao uso exclusivo de clorexidina para prevenção de pneumonia associada à ventilação mecânica: revisão sistemática e metanálise da literatura				
Área de concentração:	Clínica Odontológica Integrada				
Linha de pesquisa:	Patologia e Diagnóstico Bucal				
Projeto de Pesquisa de vinculação:	Patologia e Diagnóstico Bucal				

Reuniu-se no Anfiteatro/Sala 31 Bloco 4L - Anexo A, Campus Umuarama, da Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Odontologia, assim composta: Professores Doutores: Luciano Alberto de Castro (FOUFG); Luiz Renato Paranhos (UFU); Sérgio Vitorino Cardoso (UFU) orientador do candidato.

Iniciando os trabalhos o presidente da mesa, Dr. Sérgio Vitorino Cardoso, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovado.

Esta defesa faz parte dos requisitos necessários à obtenção do título de Mestre.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Documento assinado eletronicamente por **Sergio Vitorino Cardoso**, **Membro de Comissão**, em 13/02/2020, às 10:32, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Luiz Renato Paranhos**, **Professor(a) do Magistério Superior**, em 13/02/2020, às 10:33, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **LUCIANO ALBERTO DE CASTRO**, **Usuário Externo**, em 13/02/2020, às 10:41, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **1815783** e o código CRC **0B2E4580**.

Referência: Processo nº 23117.003049/2020-64 SEI nº 1815783

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

S586 2020

Silva, Pedro Urquiza Jayme, 1994-
Uso associado de escovação dentária e clorexidina comparado ao uso exclusivo de clorexidina para a prevenção de pneumonia associada à ventilação: revisão sistemática e metanálise de literatura [recurso eletrônico] / Pedro Urquiza Jayme Silva. - 2020.

Orientador: Sérgio Vitorino Cardoso.
Dissertação (Mestrado) - Universidade Federal de Uberlândia, Pós-graduação em Odontologia.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.di.2020.81
Inclui bibliografia.
Inclui ilustrações.

1. Odontologia. I. Cardoso, Sérgio Vitorino,1976-, (Orient.). II. Universidade Federal de Uberlândia. Pós-graduação em Odontologia. III. Título.

CDU: 616.314

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074

DEDICATÓRIA

Dedico este trabalho à minha família. Ao meu pai, por cada gota de suor derramada durante sua vida. Espero algum dia poder ser seu espelho. À minha mãe, uma leoa que travou uma grande batalha em nome da prole. Vou honrá-la em cada passo dado. À minha irmã, pelo apoio mental e espiritual. Jade realmente faz jus ao nome: rara e preciosa. Por fim, quero dedicar este trabalho à minha estrela cadente: minha avó, Dona Lourdes. Muito obrigado por apenas existir.

AGRADECIMENTOS

Aos meus colegas do Laboratório de Patologia do Hospital Odontológico / Faculdade de Odontologia, pelo apoio, cumplicidade e pela troca de conhecimento cotidiana.

Às profissionais técnicas do Laboratório de Patologia, pela ajuda na rotina laboratorial e apoio didático.

Aos professores do Laboratório de Patologia, Adriano Mota Loyola e Paulo Rogério Faria, pelas orientações acadêmicas, profissionais e pessoais, me ajudando a traçar o caminho mais correto possível.

Aos colegas de turma, pelas constantes atualizações a respeito do meio acadêmico e a amizade construída.

À minha querida amiga e companheira diária de laboratório, Anaíra Ribeiro Guedes Fonseca Costa, pelos auxílios, trocas, trabalhos, leituras, almoços e risadas.

Ao Professor Luiz Renato Paranhos, pelo auxílio na idealização e construção deste trabalho.

À minha colega de profissão e amiga Daniela Meneses Santos, pelo auxílio na construção deste trabalho.

Ao meu orientador, Professor Sérgio Vitorino Cardoso, por todos os conselhos e orientações dentro e fora do universo da pesquisa.

O terço de Fátima e o cordão de Gandhi cruzam o meu peito.

Sou como a haste fina, que qualquer brisa verga, mas nenhuma espada corta.

Maria Bethânia

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

PAV - Pneumonia Associada à Ventilação

ITRI - Infecção no Trato Respiratório Inferior

UTI - Unidade de Terapia Intensiva

CHX - Clorexidina

SDR - Síndrome do Desconforto Respiratório

RESUMO

A pneumonia associada à ventilação (PAV) é uma infecção pulmonar que se desenvolve em um paciente colocado em ventilação mecânica por mais de 48 horas. É a infecção no trato respiratório inferior mais comum em pacientes sob ventilação mecânica. A higiene oral é um importante fator na prevenção da PAV. A clorexidina é um antisséptico de efeito bacteriostático e bactericida, e seu efeito pode ser potencializadopela remoção mecânica de microrganismos através da escovação dental. O objetivo deste trabalho foi comparar a eficácia da clorexidina 0,12% versus clorexidina 0,12% associada à escovação mecânica na prevenção da PAV. Para tando, foi realizada inicialmente uma revisão sistemática de ensaios clínicos randomizados seguindo as diretrizes PRISMA-P e Cochrane. Em seguida, uma metanálise foi realizada com publicações que houvessem informado a frequência de PAV nos grupos de interesse deste estudo. Foram identificados inicialmente 2.337 trabalhos, dos quais quatro foram considerados na revisão sistemática e três na metanálise. Os estudos incluídos apresentaram baixo risco de viés. Todos os trabalhos considerados na revisão sistemática mostraram frequência menor de PAV em pacientes submetidos a protocolo de higiene oral com escovação associada à clorexidina, comparados ao uso exclusivo de clorexidina, todavia em nenhum deles essa diferença foi estatisticamente significativa. Na metanálise, os resultados agregados revelaram que o risco de PAV era 24% menor para pacientes que receberam CHX e escovação de dentes em comparação com aqueles que receberam CHX apenas, mas essa redução tampouco se mostrou estatisticamente significativa. Conclusão: Houve convergência dos resultados dos estudos quanto a efeito benéfico do uso combinado da escovação e clorexidina, todavia os resultados agregados desses estudos não resultaram em diferença significativa na frequência de PAV.

Palavras-chave:

Clorexidina, Escovação dentária, Pneumonia Associada à Ventilação.

ABSTRACT

Ventilator-associated pneumonia (VAP) is a pulmonary infection that develops in a patient on mechanical ventilation for more than 48 hours. It is the most common lower respiratory tract infection in mechanically ventilated patients. Oral hygiene is an important factor in preventing VAP. Chlorhexidine is an antiseptic with bacteriostatic and bactericidal effect, and its effect can be potentiated by mechanical removal of microorganisms through tooth brushing. The aim of this study was to compare the efficacy of 0.12% chlorhexidine versus 0.12% chlorhexidine associated with mechanical brushing to prevent VAP. However, a systematic review of randomized controlled trials was initially performed following the PRISMA-P and Cochrane guidelines. Then, a meta-analysis was performed with publications that reported the frequency of VAP in the interest groups of this study. Initially, 2,337 studies were identified, four of which were considered in the systematic review and three in the meta-analysis. The included studies had a low risk of bias. All studies considered in the systematic review showed a lower frequency of VAP in patients undergoing chlorhexidine-associated oral hygiene protocol compared to the exclusive use of chlorhexidine, but in none of them this difference was statistically significant. In the meta-analysis, aggregated results revealed that the risk of VAP was 24% lower for patients receiving CHX and tooth brushing compared with those receiving CHX alone, but this reduction was not statistically significant either. Conclusion: There was convergence of study results regarding the beneficial effect of combined use of brushing and chlorhexidine, however the aggregated results of these studies did not result in significant difference in VAP frequency.

Keywords: Chlorhexidine, Tooth Brushing, Ventilator-Associated Pneumonia.

INTRODUÇÃO E REFERENCIAL TEÓRICO

Pneumonia associada à ventilação (PAV) é um tipo de infecção pulmonar que se desenvolve em um paciente em ventilação mecânica por mais de 48 horas (Grossman & Fein, 2000). A PAV de início precoce é definida como ocorrendo nos primeiros 4 dias de hospitalização e estão associados a um melhor prognóstico. Já a PAV de início tardio engloba um período de 5 dias ou mais e estão associadas ao aumento da morbidade e mortalidade dos pacientes (American Thoracic Society, 2005).

As infecções no trato respiratório inferior (ITRIs) são as principais causas de complicações em pacientes em unidades de terapia intensiva (UTIs) (Bouadma *et al.*, 2015). As ITRIs em pacientes em UTI podem ser distinguidas de acordo com presença ou ausência de ventilação mecânica invasiva no momento de sua ocorrência (Berra *et al.*, 2003). A PAV continua sendo a ITRI mais comum em UTIs. Está relacionada a taxas de morbidade e mortalidade consideráveis (4.6%) (Spalding *et al.*, 2017). Possui taxa de incidência que varia de 10% a 20% dos pacientes que recebem mais de 48 horas de ventilação mecânica (Ego *et al.*, 2015). A etiologia bacteriana da PAV é influenciada por categoria de pacientes, tempo de início da PAV, hospitalização, antibioticoterapia prévia, tempo de internação no momento do desenvolvimento da PAV, atual antibioticoterapia para infecção concomitante e padrão local de organismos (Kalwaje *et al.*, 2018). Os agentes causadores da PAV podem variar muito diferenciando-se de acordo com regiões geográficas ou até mesmo entre diferentes hospitais do mesmo país (Melsen *et al.*, 2011; Park D, 2005).

O diagnóstico da PAV é complexo, devido a critérios que variam de acordo com a região (Ego *et al.*, 2015). Contudo, algumas características devem ser seguidas. O aparecimento de expectoração purulenta, febre (> 38 ° C), leucocitose (> 10.000 mm3) ou leucopenia (<4000 mm3), radiografia mostrando infiltrado novos e cultura da secreção respiratória (Lorente *et al.*, 2012).

Existem muitos fatores de risco associados a ocorrência da PAV. Idade avançada, predileção ao sexo masculino, aumento do tempo de ventilação mecânica, sedação, doenças associadas ao coração e pulmões, regurgitação, aspiração, queimaduras, antibioticoterapia prévia, operações invasivas e polimorfismos genéticos (Metheny *et al.*, 2011; Wei &Yang, 2019; Wu *et al.*, 2019).

Várias diretrizes internacionais sobre prevenção da PAV estão disponíveis (Rebmann & Greene, 2010; Xie *et al.*, 2019). Os pontos citados como mais relevantes na prevenção da PAV se relacionam a minimização do tempo de exposição à ventilação mecânica invasiva (Klompas, 2015) e os cuidados intensos em higiene oral (Soh *et al.*, 2011). Quanto à minimização do tempo de exposição à ventilação mecânica invasiva, o uso de abordagens não invasivas de ventilação é incentivado, utilizando, por exemplo, a pressão positiva de duas vias ou pressão positiva contínua (Xie *et al.*, 2019) para minimizar sua duração. Protocolos de desmame do ventilador, como a interrupção diária da sedação e coordenação com um teste de respiração espontânea (Bouadma *et al.*, 2012) ou protocolos de cuidados baseados em evidências também podem ser eficazes para encurtar duração da ventilação mecânica (Alcan *et al.*, 2016).

Quanto à higiene oral, estudos pregressos geraram evidências de sua importância nos cuidados para prevenção da PAV (Conley *et al.*, 2013; McCue *et al.*, 2019). A colonização oral é uma patogênese conhecida da PAV (Jackson & Owens; 2019). A placa dental e a colonização de microrganismos são significativas, a partir do 4º dia de intubação conferindo um maior risco de PAV (Munro *et al.*, 2006). No passado, a colonização oral foi tratada com antibióticos intraorais. Contudo, um aumento na resistência a antibióticos resultou em seu desuso, sendo necessária a consideração de medicamentos alternativos (Jackson & Owens; 2019). Em 2012, o Institute for Healthcare Improvement recomendou a inclusão do cuidado bucal diário com CHX para pacientes em ventilação mecânica devido às suas propriedades inibitórias na formação de placa dental e problemas gengivais e com base em evidências (Institute for Healthcare Improvement, 2012).

A CHX é um antisséptico de amplo espectro essencial para a saúde em todo o mundo (Institute for Healthcare Improvement, 2012). Ela apresenta efeito

bacteriostático, atuando na inibição do crescimento bacteriano. Possui também efeito bactericida, que resulta na morte das bactérias (Kumar *et al.*,2017). A CHX é uma biguanida catiônica que se liga as paredes celulares bacterianas, alterando seu equilíbrio osmótico (Septimus & Schweizerc., 2016). A ação da CHX dar-se-á pela interação com a célula ocasionando uma desorientação da membrana lipoproteica, uma vez que a CHX apresenta em sua composição grupos lipofílicos (Hugo *et al.*, 1964).

Como enxaguante bucal, a CHX pode reduzir as colonizações orais (Ellepola *et al.*, 2013; Jackson & Owens; 2019). Entretanto, embora o controle farmacológico da placa dental, por meio do uso de CHX é prática e amplamente aceita entre os profissionais de saúde (Vidal *et al.*, 2017), a abordagem química contra o acumulado de placa é marginal, uma vez que a placa atua como um biofilme em qual a bactéria é consideravelmente menos sensível à antibioticoterapia quando comparada a sua forma planctônica (ten Cate, 2006).

Uma alternativa de potencialização do efeito da CHX é a remoção mecânica de microrganismos, que pode aumentar a eficácia dos efeitos da CHX em bactérias remanescentes ou em crescimento bacteriano (Kishimoto & Urade, 2007). Escovas de dente são consideradas a melhor ferramenta para cuidados bucais mecânicos em uma população saudável (Chacko *et al.*, 2017). Estudos demonstraram que para controlar as bactérias da orofaringe é necessário a remoção da placa dental, sendo a escovação a melhor maneira de remover (El-Solh *et al.*, 2004; Shinn, 2004). Outros estudos ainda indicaram que cuidados bucais com um agente antisséptico mais escovação dental reduziam a incidência de PAV em comparação com a ausência dos mesmos (Mori *et al.*, 2006; Hutchins *et al.*, 2009; Sona *et al.*, 2009).

Existem vários estudos envolvendo cuidados orais e prevenção da PAV. ainda não há um consenso quanto à melhor técnica, a frequência a ser utilizada e, a escolha do agente farmacológico e sua concentração (Chacko *et al.*, 2017, Tantipong *et al.*, 2008; Long *et al.*, 2012; Vidal *et al.*, 2017; Atashi *et al.*, 2018). Em um estudo transversal de Miranda et al. (2016), onde foram avaliados os métodos utilizados nos cuidados bucais de pacientes em UTIs, observou-se que apenas a CHX a 0,12% era utilizada em 49,3% das respostas dos profissionais de enfermagem para

higienização da cavidade oral, enquanto a associação da CHX a 0,12% com a escova de dentes era 11,3% (Miranda *et al.*, 2016).

É-se comprovado que o uso de CHX em altas concentrações pode causar efeitos adversos. Irritações na mucosa oral (Tantipong *et al.*, 2008) e o desenvolvimento da SDR pela sua ingestão (Cutts *et al.*, 2017) complicações relatadas na literatura. A SDR é descrita como uma sequela de reações externas, associada a lesões difusas alveolares e endoteliais (Cutts *et al.*, 2017) e que em pacientes fragilizados pode ser fatal (Hirata & Kurokawa, 2002). Modelos em animais mostraram que concentrações sistêmicas mais altas de CHX induziram inflamação aguda no pulmão, com hemorragias perivasculares e interalveolares, congestão capilar e edema alveolar, além de alterações comportamentais semelhantes às da SDR (Orito *et al.*, 2006; Xue *et al.*, 2011).

O objetivo deste estudo é realizar uma revisão sistemática da literatura para avaliar a eficácia da CHX 0,12% versus CHX 0,12% com escovação mecânica na redução do risco da PAV em adultos que necessitam de ventilação mecânica em UTIs.

CAPÍTULO ÚNICO

Silva PUJ, Santos DM, Macedo DR, Paranhos LR, Cardoso SV. Combination of tooth brushing and chlorhexidine to reduce the risk of ventilator-associated pneumonia compared to using only chlorhexidine: a systematic review with meta-analysis.

Este trabalho será submetido à revista Plos One Medicine.

Combination of tooth brushing and chlorhexidine compared to exclusive use of chlorhexidine to reduce the risk of ventilator-associated pneumonia: a systematic review with meta-analysis

Pedro Urquiza Jayme Silva[1], Daniela Meneses[2], Dhiancarlo Rocha Macedo[3], Cauane Blumenberg[4], Luiz Renato Paranhos[5], Sérgio Vitorino Cardoso[1,✉]

1. Graduate Program in Dentistry, School of Dentistry, Federal University of Uberlandia, Brazil.
2. Resident Program of Oral and Maxillofacial Surgery and Traumatology. School of Medicine, Federal University of Uberlandia, Brazil.
3. Dental Hospital, Federal University of Uberlandia, Brazil.
4. Post-graduate Program in Epidemiology. Faculty of Medicine. Federal University of Pelotas. Pelotas / RS, Brazil.
5. Area of Preventive and Social Dentistry, School of Dentistry, Federal University of Uberlandia, Brazil.

✉ Corresponding author: **Sérgio Vitorino Cardoso**

Universidade Federal de Uberlândia

Faculdade de Odontologia, Área de Patologia

Av. Pará, 1720 – Campus Umuarama

ZIP-Code: 38400-902

sv.cardoso@ufu.br

Abstract

Ventilator-associated pneumonia (VAP) is a pulmonary infection that develops in a patient on mechanical ventilation for more than 48 hours. It is the most common lower respiratory tract infection in mechanically ventilated patients. Oral hygiene is an important factor in preventing VAP. Chlorhexidine is an antiseptic with bacteriostatic and bactericidal effect, and its effect can be potentiated by mechanical removal of microorganisms through toothbrushing. The aim of this study was to compare the efficacy of 0.12% chlorhexidine versus 0.12% chlorhexidine associated with mechanical brushing to prevent VAP. However, a systematic review of randomized controlled trials was

initially performed following the PRISMA-P and Cochrane guidelines. Then, a meta-analysis was performed with publications that reported the frequency of VAP in the interest groups of this study. Initially, 2,337 studies were identified, four of which were considered in the systematic review and three in the meta-analysis. The included studies had a low risk of bias. All studies considered in the systematic review showed a lower frequency of VAP in patients undergoing chlorhexidine-associated oral hygiene protocol compared to the exclusive use of chlorhexidine, but in none of them this difference was statistically significant. In the meta-analysis, aggregated results revealed that the risk of VAP was 24% lower for patients receiving chlorhexidine and toothbrushing compared with those receiving CHX alone, but this reduction was not statistically significant either. Conclusion: There was convergence of study results regarding the beneficial effect of combined use of brushing and chlorhexidine, however the aggregated results of these studies did not result in significant difference in VAP frequency.

Keywords: Chlorhexidine, Tooth brushing, Ventilator-Associated Pneumonia.

Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs more than 48 hours after the onset of mechanical ventilation [1]. It affects 10% to 20% of patients who receive more than 48 hours of mechanical ventilation. The diagnosis of VAP requires the appearance of purulent sputum, fever (> 38°C) or hypothermia (< 35.5ºC), leukocytosis (> 10,000mm^3) or leukopenia (<4000mm^3), positive bacterial culture of respiratory secretion (> 10^6cfu/mL) , and radiograph showing additional or progressive pulmonary infiltrates [2].

There are many risk factors associated with VAP, such as older age, male gender, increased time on mechanical ventilation, sedation, heart and lung disease, regurgitation, aspiration, prior antibiotic therapy and invasive operations [3]. Burns are also a risk factor for VAP through pulmonary inflammation from direct lung injury or systemic immune dysfunction [4]. Genetic polymorphism related to inflammatory mediators also may cause an increased risk of developing VAP, possibly due to ineffective response to bacteria [5].

Oral hygiene by means of a variety of procedures is an important measure to prevent VAP [6]. For instance, aspiration of secretions, toothbrushing or dental and mucosal cleansing with chlorhexidine (CHX) may reduce the risk of VAP [7]. CHX is a cationic biguanide that binds to bacterial cell walls, thus impairing and even perforating the lipoproteic membranes [8,9] with bacteriostatic and bactericidal effect, which relates to concentration of the substance [10]. Its use for oral hygiene in patients under mechanical ventilation reduces the risk of VAP [11-14]. As a mouthwash, CHX reduces bacterial colonization in the oral cavity [15,16]. However, the presence of a biofilm in the surface of the teeth limits the action of any mouthwash [17]. So, previous mechanical disruption of dental biofilm through toothbrushing improves the effect of CHX [18-20] and could therefore increases the benefit in preventing VAP [20-22].

High chlorhexidine concentrations are associated with adverse effects [23]. Dental discoloration and oral mucosa irritation were effects attributed to the use of 0.2% and 2% CHX [24]. Lesions in the oral mucosa were also identified. Erosive lesions, ulcerations, white / yellow plaque formation, and mucosal bleeding have been observed in patients in intensive care units [25]. CHX 0.12% was effective in preventing VAP in surgical patients [26].

The aim of the present study was to compare the reduction of the risk of VAP with the use of oral 0.12% chlorhexidine combined with toothbrushing versus exclusive use of 0.12% chlorhexidine in the prevention of VAP through systematic review and meta-analysis.

Material and methods

Protocol and registration

This systematic review was performed according to the PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) recommendations [27] and the Cochrane guidelines [28]. The systematic review protocol was registered into the PROSPERO database (CRD *[Blinding]*).

Study design and eligibility criteria

This study was a systematic review followed by a metanalysis based on the PICO strategy, aiming to answer the following review question: “Is toothbrushing combined with the use of 0.12% CHX (intervention) in patients undergoing mechanical ventilation (population) more effective for preventing VAP (outcome) than using only CHX (comparation)?”
The inclusion criteria were randomized controlled trials that compared oral hygiene using 0.12% CHX with or without toothbrushing in patients undergoing invasive (tracheal) mechanical ventilation. There was no restriction of year or status of publication (published, accepted/ahead of print articles).
Exclusion criteria were studies not related to the objective of the present study, participants with less than 18 years of age, non-original works (review articles, editorials, books/book chapters), or papers with insufficient data (letters, personal opinions, conference abstracts).

Sources of information and search

All steps were performed to minimize selection and publication biases. The PubMed (including MedLine), Scopus, Embase, SciELO, Web of Science, Latin-American and Caribbean Health Sciences Literature (LILACS), Cochrane and LIVIVO databases were used as primary study sources. OpenGrey and Open Access Thesis and Dissertations (OATD) were used to access the "gray literature" to avoid bias regarding the lack of published negative results. (Figure 1). The MeSH (Medical Subject Headings), DeCS (Health Sciences Descriptors), and Emtree (Embase Subject Headings) resources were used to select the search descriptors. The Boolean operators "AND" and "OR" were used to enhance the research strategy through several combinations. The search strategy is detailed in Table 1. The bibliographic research was performed in November 2019. The results obtained were imported into the EndNote Web™ software (Thomson Reuters, Toronto, Canada for removal of duplicates. The remaining results were imported into Microsoft Word™ 2010 (Microsoft™ Ltd, Washington, USA), in which the remaining duplicates were manually removed.

Study selection

Two independent reviewers [Blinding] were previously calibrated in a sample of 20% of the studies and reached adequate inter-examiner agreement. The eligibility review was then performed independently by these reviewers, with disagreements solved by discussion with a third reviewer [Blinding] to reach a consensus.

The selection of the studies was performed in two stages. The first stage comprised a thorough analysis of the titles and abstracts of the articles. Reviewers were not blinded to the names of authors and journals. Studies with titles not related to the topic of interest of our review were eliminated in this stage. Titles that met the objectives of our study but did not have abstracts available were fully analyzed in the second stage. In the last stage the eligible studies had their full texts obtained and evaluated to verify whether they fulfilled the eligibility criteria. The references of the eligible articles were carefully assessed to check for studies that were not detected in the main search strategy. Excluded studies were registered separately along with the reasons for exclusion.

Data collection

After selection, studies were analysed by two reviewers [*Blinding*], who extracted the following information from the articles: identification (author, year, country and research location), sample characteristics (number of patients in each study, gender distribution, mean age, and the Acute Physiology and Chronic Health assessment – APACHE [29,30] and main results (ventilation time, microbiota assessment, VAP incidence, mortality, and conclusions).

In order to ensure the consistency, the reviewers [*Blinding*] extracted the information jointly from an eligible study. Any disagreement was solved through discussion, and when both reviewers disagreed, a third one [*Blinding*] was consulted to make a final decision.

Risk of individual bias of the studies

The Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for randomized controlled trials [31] was used to assess the risk of bias and the individual quality of the studies selected. Two authors [*Blinding*] assessed independently each domain regarding their potential risk of bias, as recommended by the PRISMA-P statement [27]. Each study was categorized according to the percentage of positive

answers to the questions corresponding to the assessment tool. Risk of bias was considered ***High*** when the study obtained 49% of "yes" answers, ***Moderate*** when the study obtained 50% to 69% of "yes" answers, and ***Low*** when the study reached more than 70% of "yes" score.

Summary measures and syntheses of results (Metanalysis)

Eligible studies that provided sufficient information to calculate the relative risk (RR) of VAP for patients that received 0.12% CHX combined with toothbrushing compared to patients that received only 0.12% CHX were included in the statistical analyses. A metanalysis using random effects model was adjusted in order to estimate a combined RR. The heterogeneity between the studies was evaluated using the i^2 statistic. A 5% significance level was considered for the statistical analyses.

Quality of evidence collection

Quality of evidence and strength of recommendation were assessed with the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) tool with the GRADE pro GDT software (http://gdt.guidelinedevelopment.org) [32]. This assessment was based on study design, methodological limitations, inconsistency, indirect evidence, imprecision, and other considerations. The level of certainty among the identified evidence was characterized as high, moderate, low, or very low [32].

Results

Study selection

During the first phase of study selection, 2,337 works were found. No objective-related studies were found in the “gray literature”. After removing duplicates, 1,071 papers remained for the analysis of titles and abstracts. After a detailed analysis, only four studies were eligible for the full text review. The references of these eligible studies were evaluated carefully, and no additional article was selected. None of these four studies

were excluded for the purpose of qualitative analysis (review). Fig. 1 reproduces the process of search, identification, inclusion, and exclusion of articles.

Study characteristics of eligible studies

The studies were published between 2009 and 2017 and were performed in the United States [11], Spain [2,33] and Brazil [34]. Sources of information regarding demographic and clinical characteristics of the population are available in Table 2. All articles were approved by the Ethics Committee of their respective institution or hospital and also reported that informed consents were obtained prior to the start of the study. No studies followed the CONSORT statement. Half of the included studies reported calibration among nurses [33] and dentists [34] who performed oral hygiene procedures. Two studies [33,34] presented the registration number of their randomized controlled clinical trial.

Risk of individual bias of the studies

Table 3 shows the risk of bias and individual quality of the studies included in this systematic review. All studies [2,11,33,34] presented low risk of bias. One study [2] did not provide details about the randomization procedure and allocation concealment. No study was blinded because the participants were admitted to intensive care units under invasive mechanical ventilation in unconscious state. The questions Q.5 and Q.6 were considered "unclear" for three studies [2,11,33] because it was not clear if those delivering treatment were aware of the allocation of the participants. The question Q.7 was considered "no" for two studies [11,34] because the groups were not treated identically according to the intervention of interest.

Outcomes of each study

The VAP incidence rate was mentioned for all but one study, with the exception [11] comparing groups with inadequate features to our question. Other outcomes common to two or more studies were reported on Table 4. Three studies reported the average days of ventilation [2,33,34]. All studies presented positive microbiological tests for VAP identification. None of the studies reported mortality rate.

Synthesis of meta-analysis

Of the four eligible studies of the systematic review, one [11] was not included due to the lack of comparison between intervention and control groups. The summary results of the remaining three studies included in the metanalysis [2,33,34], as shown in the Figure 2, evidenced a 24% reduction in the relative risk of VAP for patients submitted to CHX + toothbrushing, but this effect did not reach statistical significance.

Quality of evidence collection

The quality of evidence from the outcome evaluated by the GRADE tool[19] was assessed as "Moderate", which means. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Table 5 shows more details about the evaluation of each GRADE tool domain.

Discussion

This systematic review of the literature aimed to evaluate the effectiveness of CHX 0.12% versus CHX 0.12% with toothbrushing in reducing the risk of VAP in adults requiring mechanical ventilation in intensive care units. The results found in the meta-analysis showed a non-significant reduction in the incidence of VAP of the CHX + Toothbrushing group compared to the group that used only CHX. This reduction in incidence suggest a protective effect of toothbrushing associated with CHX use in relation to VAP but must be interpreted with great caution.

The oral cavity microbiota is highly diverse and dynamic, mainly due to the wide variety of microbial habitats in the mouth and the changes that can arise in these environments due to changes in diet, salivary flow and oral hygiene interventions [35-39]. The oral cavity is directly linked to the lower airways, so associations between oral microbiology and respiratory infections are often made [40]. Carrilho-Neto et al. (2011) showed a reduction in oral hygiene for most hospitalized patients, reporting a positive correlation

between dental plaque index and gingival inflammation index [41]. Gingival inflammation caused by poor oral hygiene in intubated patients can also cause lung inflammation [41-43].

Dental plaque and colonization of microorganisms are significant from the 4th day of intubation conferring a higher risk of VAP [44]. A study by Sands et al. (2017) revealed that the composition of dental plaque in mechanically ventilated patients, in a significant proportion (approximately one third), included possible respiratory pathogens *S. aureus and P. aeruginosa* [40]. This information can be corroborated in one of the eligible studies. Pobo et al. (2009) reported the association of the pathogens *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae* in their study [33]. In another study by Sandes et al. (2016), in addition to the presence of *Staphylococcus aureus*, were identified strains of *Haemophilus influenzae*, an organism associated with respiratory infection [45]. Although an opportunistic pathogen, *Haemophilus influenzae* is a common nasopharynx diner [45,46].

CHX has a bacteriostatic effect, inhibiting bacterial growth and a bactericidal effect that results in bacterial death - the mechanism of action being dependent on solution concentration [10]. CHX is a cationic biguanide that binds to bacterial cell walls, altering their osmotic balance [8]. The action of CHX will occur by interaction with the cell causing a disorientation of the lipoprotein membrane, since CHX presents in its composition lipophilic groups [9]. In addition to CHX's bactericidal and bacteriostatic potential, toothbrushing has shown promising effects on VAP [47,48]. Disorganization of plaque or biofilm that is adhered to the dental surface can be performed mechanically and chemically [49]. Brushing assists in the removal of biofilm through brush bristles, as mechanical contact can break plaque that is adherent to the tooth surface [50,51].

Meinberg et al. (2012) conducted a clinical trial using CHX 2% with and without toothbrushing, where the incidence of VAP was 55.8% [12]. Compared to the VAP incidence rates among the eligible studies of the present review [2,33,34], there is a reduction in VAP development. This result helps support the use of CHX 0.12% in VAP prevention care in mechanically ventilated patients. In addition, the use of CHX at high concentrations may cause adverse effects such as oral mucosal irritations [52] and the development of respiratory distress syndrome (RDS) by ingestion [53]. RDS is described as a sequela of external reactions, associated with diffuse alveolar and endothelial lesions [54] and which in fragile patients can be fatal [55].

VAP is associated with increased length of stay in mechanical ventilation related to high patient morbidity and mortality rates, as well as increased hospital costs [55,56]. In any of the eligible papers included in the present systematic review, the comparison between toothbrushing combined with CHX 0.12% and CHX 0.12% showed no significant reduction in mean days of mechanical ventilation [2,33]. This result may show that the length of stay in mechanical ventilation is a risk factor that overlaps the VAP prevention protocol. This relationship can be identified in one of the eligible studies, in which the majority of VAP cases occurred after the fourth day of mechanical ventilation [34].

Among eligible articles, it is possible to observe that addition of toothbrushing is not more effective in preventing VAP than solely rinsing 0.12% chlorexidine [12,33]. Manual brushing added to CHX use does not help prevent VAP among patients on intensive mechanical ventilation therapy [2]. However, in the meta-analysis results of this study, there was a 24% reduction in the incidence of VAP in the CHX 0.12% + toothbrushing group. This result may demonstrate the protective but supporting role of brushing in preventing VAP. This conclusion can be corroborated by a study by Yao et al (2011) that assessed the risk of VAP using toothbrushing + purified water, showing that the incidence of the disease was 34% [57]. Among eligible studies, the incidence of VAP ranged from 10.32% [2] to 22.4% [33].

VAP is associated with considerable mortality rates (Wu et al., 2019). A review by Wilhemina et al. (2011) showed that attributable mortality resulting from VAP is approximately 10%, ranging from 3% to 17% in subgroup analyzes [59]. Corrado et al. (2017) evidences in a retrospective analysis a mortality rate attributed to VAP of 21.6% of patients [60]. Eligible studies of the present work did not bring VAP mortality rates. This may represent an important limitation since the population maintained on mechanical ventilation is in critical condition. The cause of death may be related to previous morbidity [58].

This systematic review and meta-analysis have some limitations. The first is the low number of studies included. Second, eligible studies showed a lack of information collected, such as patient mortality and overall length of stay in intensive care units. Thus, our results should be interpreted with caution and further studies with a standardized design are encouraged to confirm the use of 0.12% CHX + toothbrushing in reducing the risk of VAP in patients undergoing mechanical ventilation in intensive care units. As a strength, our review had a very comprehensive search strategy, including part of the gray

literature, and is the first meta-analysis of clinical trials comparing the CHX 0.12% + toothbrushing and CHX 0.12% protocols.

Healthcare professionals should be aware of the benefits of oral hygiene in intensive care unit patients in order to reduce the incidence of VAP. The adoption of CHX may represent an improvement in the mortality rate of patients on mechanical ventilation and consequently an improvement in the patients' quality of life, as well as a reduction in hospital expenses. Future research should focus on a single VAP prevention protocol using CHX + brushing, as well as improved data collection on total hospital length of stay and mortality rate, and an increase in sample size.

Conclusions

Based on a moderate level of evidence, it was concluded that addition of toothbrushing to cleansing with 0.12% CHX in oral care of patients under mechanical ventilation does not significantly reduces the risk of VAP.

References

[1] Grossman RF, Fein A. Evidence-based assessment of diagnostic tests for ventilator-associated pneumonia. Executive summary. *Chest*. 2000;117(4 Suppl 2):177S–181S. doi:10.1378/chest.117.4_suppl_2.177s

[2] Lorente L, Lecuona M, Jiménez A, et al. Ventilator-associated pneumonia with or without toothbrushing: a randomized controlled trial. *Eur J Clin Microbiol Infect Dis*. 2012;31(10):2621–2629. doi:10.1007/s10096-012-1605-y

[3] Wu D, Wu C, Zhang S, Zhong Y. Risk Factors of Ventilator-Associated Pneumonia in Critically III Patients. *Front Pharmacol*. 2019;10:482. doi:10.3389/fphar.2019.00482

[4] Sen S, Johnston C, Greenhalgh D, Palmieri T. Ventilator-Associated Pneumonia Prevention Bundle Significantly Reduces the Risk of Ventilator-Associated Pneumonia in Critically Ill Burn Patients. *J Burn Care Res*. 2016;37(3):166–171. doi:10.1097/BCR.0000000000000228

[5] Kotsaki A, Raftogiannis M, Routsi C, et al. Genetic polymorphisms within tumor necrosis factor gene promoter region: a role for susceptibility to ventilator-associated pneumonia. *Cytokine*. 2012;59(2):358–363. doi:10.1016/j.cyto.2012.04.040

[6] Wise MP, Williams DW. Oral care and pulmonary infection - the importance of plaque scoring. *Crit Care*. 2013;17(1):101. doi:10.1186/cc11896

[7] Hua F, Xie H, Worthington HV, Furness S, Zhang Q, Li C. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. *Cochrane Database Syst Rev*. 2016;10(10):CD008367. doi:10.1002/14651858.CD008367.pub3

[8] Septimus JE, Schweizerc ML. Decolonization in Prevention of Health Care-Associated Infections Clinical Microbiology Reviews. 2016; 29(2). https://doi.org/10.1128/CMR.00049-15

[9] Hugo WB, Longworth AR. Some aspects of the mode of action of chlorhexidine. *J Pharm Pharmacol*. 1964;16:655–662.

doi:10.1111/j.2042-7158.1964.tb07384.x

[10] Shrada B, Kumar J?. Chlorhexidine Mouthwash- A Review. *J. Pharm. Sci. & Res*. 2017; 9(9): 1450-1452.

[11] Munro CL, Grap MJ, Jones DJ, McClish DK, Sessler CN. Chlorhexidine, toothbrushing, and preventing ventilator-associated pneumonia in critically ill adults. *Am J Crit Care*. 2009;18(5):428–438. doi:10.4037/ajcc2009792

[12] Meinberg MC, Cheade MF, Miranda AL, Fachini MM, Lobo SM. The use of 2% chlorhexidine gel and toothbrushing for oral hygiene of patients receiving mechanical ventilation: effects on ventilator-associated pneumonia. *Rev Bras Ter Intensiva*. 2012;24(4):369–374. doi:10.1590/s0103-507x2012000400013

[13] Sebastian MR, Lodha R, Kapil A, Kabra SK. Oral mucosal decontamination with chlorhexidine for the prevention of ventilator-associated pneumonia in children - a randomized, controlled trial. *Pediatr Crit Care Med*. 2012;13(5):e305–e310. doi:10.1097/PCC.0b013e31824ea119

[14] Cabov T, Macan D, Husedzinović I, et al. The impact of oral health and 0.2% chlorhexidine oral gel on the prevalence of nosocomial infections in surgical intensive-care patients: a randomized placebo-controlled study. *Wien Klin Wochenschr*. 2010;122(13-14):397–404. doi:10.1007/s00508-010-1397-y

[15] Ellepola AN, Joseph BK, Khan ZU. Changes in the cell surface hydrophobicity of oral Candida albicans from smokers, diabetics, asthmatics, and healthy individuals following limited exposure to chlorhexidine gluconate. *Med Princ Pract*. 2013;22(3):250–254. doi:10.1159/000345641

[16] Jackson L, Owens M. Does oral care with chlorhexidine reduce ventilator-associated pneumonia in mechanically ventilated adults?. *Br J Nurs*. 2019;28(11):682–689. doi:10.12968/bjon.2019.28.11.682

[17] ten Cate JM. Biofilms, a new approach to the microbiology of dental plaque. *Odontology*. 2006;94(1):1–9. doi:10.1007/s10266-006-0063-3

[18] Chacko, R., Rajan, A., Lionel, P., Thilagavathi, M., Yadav, B., & Premkumar, J. (2017). Oral decontamination techniques and ventilator-associated pneumonia. *British Journal of Nursing,* 26(11): 594–599.

doi:10.12968/bjon.2017.26.11.594

[19] Kishimoto H, Urade M. Mechanical tooth cleaning before chlorhexidine application. *Am J Respir Crit Care Med*. 2007;175(4):418. doi:10.1164/ajrccm.175.4.418a

[20] Mori H, Hirasawa H, Oda S, Shiga H, Matsuda K, Nakamura M. Oral care reduces incidence of ventilator-associated pneumonia in ICU populations. *Intensive Care Med*. 2006;32(2):230–236.
doi:10.1007/s00134-005-0014-4

[21] Hutchins K, Karras G, Erwin J, Sullivan KL. Ventilator-associated pneumonia and oral care: a successful quality improvement project. *Am J Infect Control*. 2009;37(7):590–597.
doi:10.1016/j.ajic.2008.12.007

[22] Sona CS, Zack JE, Schallom ME, et al. The impact of a simple, low-cost oral care protocol on ventilator-associated pneumonia rates in a surgical intensive care unit. *J Intensive Care Med*. 2009;24(1):54–62.
doi:10.1177/0885066608326972

[23] Ferretti GA, Brown AT, Raybould TP, Lillich TT. Oral antimicrobial agents--chlorhexidine. *NCI Monogr*. 1990;(9):51–55.

[24] Zand F, Zahed L, Mansouri P, Dehghanrad F, Bahrani M, Ghorbani M. The effects of oral rinse with 0.2% and 2% chlorhexidine on oropharyngeal colonization and ventilator associated pneumonia in adults' intensive care units. *J Crit Care*. 2017;40:318–322.
doi:10.1016/j.jcrc.2017.02.029

[25] Plantinga NL, Wittekamp BHJ, Leleu K, et al. Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU. *Intensive Care Med*. 2016;42(4):620–621. doi:10.1007/s00134-016-4217-7

[26] Nicolosi LN, del Carmen Rubio M, Martinez CD, González NN, Cruz ME. Effect of oral hygiene and 0.12% chlorhexidine gluconate oral rinse in preventing ventilator-associated pneumonia after cardiovascular surgery. *Respir Care*. 2014;59(4):504–509.

doi:10.4187/respcare.02666

[27] Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4(1):1. doi:10.1186/2046-4053-4-1

[28] Higgins JP, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928. doi:10.1136/bmj.d5928

[29] Wang Y, Xiao QM, Qi HN, et al. Zhonghua Lao Dong Wei. *Sheng Zhi Ye Bing Za Zhi*. 2019;37(1):43–45. doi:10.3760/cma.j.issn.1001-9391.2019.01.009

[30] Fortis S, O'Shea AMJ, Beck BF, et al. An automated computerized critical illness severity scoring system derived from APACHE III: modified APACHE. *J Crit Care*. 2018;48:237–242. doi:10.1016/j.jcrc.2018.09.005

[31] Tufanaru C, Munn Z, Aromataris E, Campbell J, Hopp L. Chapter 3: Systematic reviews of effectiveness. In: Aromataris E, Munn Z (Editors). *Joanna Briggs Institute Reviewer's Manual*. The Joanna Briggs Institute, 2017. Available from https://reviewersmanual.joannabriggs.org/

[32] Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J *et al* (2011) GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol 64:401–406 20. https://doi.org/10.1016/j.jclinepi.2010.07.015.

[33] Pobo A, Lisboa T, Rodriguez A, et al. A randomized trial of dental brushing for preventing ventilator-associated pneumonia. *Chest*. 2009;136(2):433–439. doi:10.1378/chest.09-0706

[34] de Lacerda Vidal CF, Vidal AK, Monteiro JG Jr, et al. Impact of oral hygiene involving toothbrushing versus chlorhexidine in the prevention of ventilator-associated pneumonia: a randomized study [published correction appears in BMC Infect Dis. 2017 Feb 27;17 (1):173]. *BMC Infect Dis*. 2017;17(1):112. doi:10.1186/s12879-017-2188-0

[35] Dennesen P, van der Ven A, Vlasveld M, et al. Inadequate salivary flow and poor oral mucosal status in intubated intensive care unit patients. *Crit Care Med*. 2003;31(3):781–786.

doi:10.1097/01.CCM.0000053646.04085.29

[36] Marsh PD. Controlling the oral biofilm with antimicrobials. *J Dent*. 2010;38 Suppl 1:S11–S15.
doi:10.1016/S0300-5712(10)70005-1

[37] Tada A, Hanada N. Opportunistic respiratory pathogens in the oral cavity of the elderly. *FEMS Immunol Med Microbiol*. 2010;60(1):1–17.
doi:10.1111/j.1574-695X.2010.00709.x

[38] Wade WG. The oral microbiome in health and disease. *Pharmacol Res*. 2013;69(1):137–143.
doi:10.1016/j.phrs.2012.11.006

[39] Wise MP, Cole JM, Williams DW, Lewis MA, Frost PJ. Efficacy of oral chlorhexidine in critical care. *Crit Care*. 2008;12(3):419.
doi:10.1186/cc6886

[40] Sands KM, Wilson MJ, Lewis MAO, et al. Respiratory pathogen colonization of dental plaque, the lower airways, and endotracheal tube biofilms during mechanical ventilation. *J Crit Care*. 2017;37:30–37.
doi:10.1016/j.jcrc.2016.07.019

[41] Carrilho Neto A, De Paula Ramos S, Sant'ana AC, Passanezi E. Oral health status among hospitalized patients. *Int J Dent Hyg*. 2011;9(1):21–29.
doi:10.1111/j.1601-5037.2009.00423.x

[42] Eddens T, Kolls JK. Host defenses against bacterial lower respiratory tract infection. *Curr Opin Immunol*. 2012;24(4):424–430.
doi:10.1016/j.coi.2012.07.005

[43] Hunter JD. Ventilator associated pneumonia. *Postgrad Med J*. 2006;82(965):172–178.
doi:10.1136/pgmj.2005.036905

[44] Munro CL, Grap MJ, Elswick RK Jr, McKinney J, Sessler CN, Hummel RS 3rd. Oral health status and development of ventilator-associated pneumonia: a descriptive study. *Am J Crit Care*. 2006;15(5):453–460.

[45] Sands KM, Twigg JA, Lewis MAO, et al. Microbial profiling of dental plaque from mechanically ventilated patients. *J Med Microbiol*. 2016;65(2):147–159. doi:10.1099/jmm.0.000212

[46] King P. Haemophilus influenzae and the lung (Haemophilus and the lung). *Clin Transl Med*. 2012;1(1):10. Published 2012 Jun 14. doi:10.1186/2001-1326-1-10

[47] Russell AD. Chlorhexidine: Antibacterial action and bacterial resistance. *Infection*. 1986;14:212–5.

[48] Roberts N & Moule P. Chlorhexidine and tooth-brushing as prevention strategies in reducing ventilator-associated pneumonia rates. *Nursing in Critical Care.* 2011; 16(6), 295–302.
doi:10.1111/j.1478-5153.2011.00465.x

[49] Palmier TR & Fonseca TFR. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control?. *Braz. Oral Res*. 2009; 23(1):39-48.
http://dx.doi.org/10.1590/S180683242009000500007.

[50] Chandki R, Banthia P, Banthia R. Biofilms: A microbial home. *J Indian Soc Periodontol*. 2011;15(2):111–114.

doi:10.4103/0972-124X.84377

[51] Verkaik MJ, Busscher HJ, Rustema-Abbing M, Slomp AM, Abbas F, van der Mei HC. Oral biofilm models for mechanical plaque removal. *Clin Oral Investig*. 2010;14(4):403–409.

doi:10.1007/s00784-009-0309-x

[52] Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V. Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. *Infect Control Hosp Epidemiol*. 2008;29(2):131–136.
doi:10.1086/526438

[53] Hirata K, Kurokawa A. Chlorhexidine gluconate ingestion resulting in fatal respiratory distress syndrome. *Vet Hum Toxicol*. 2002;44(2):89–91.

[54] Cutts S, Talboys R, Paspula C, Prempeh EM, Fanous R, Ail D. Adult respiratory distress syndrome. *Ann R Coll Surg Engl*. 2017;99(1):12–16. doi:10.1308/rcsann.2016.0238

[55] Ferreira CR, de Souza DF, Cunha TM, Tavares M, Reis SSA, Pedroso RS, Röder DVDB. The effectiveness of a bundle in the prevention of ventilator-associated pneumonia. *The Brazilian Journal of Infectious Diseases,* 2016; 20(3), 267–271. doi:10.1016/j.bjid.2016.03.004

[56] Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. *Am J Respir Crit Care Med*. 1999;159(4 Pt 1):1249–1256. doi:10.1164/ajrccm.159.4.9807050

[57] Yao LY, Chang CK, Maa SH, Wang C, Chen CC. Brushing teeth with purified water to reduce ventilator-associated pneumonia. *J Nurs Res*. 2011;19(4):289–297. doi:10.1097/JNR.0b013e318236d05f

[58] Wu D, Wu C, Zhang S, Zhong Y. Risk Factors of Ventilator-Associated Pneumonia in Critically III Patients. *Front Pharmacol*. 2019;10:482. doi:10.3389/fphar.2019.00482

[59] Melsen WG, Rovers MM, Koeman M, Bonten MJ. Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies. *Crit Care Med*. 2011;39(12):2736–2742. doi:10.1097/CCM.0b013e3182281f33

[60] Corrado RE, Lee D, Lucero DE, Varma JK, Vora NM. Burden of Adult Community-acquired, Health-care-Associated, Hospital-Acquired, and Ventilator-Associated Pneumonia: New York City, 2010 to 2014. *Chest*. 2017;152(5):930–942. doi:10.1016/j.chest.2017.04.162

Figures and Tables

Table 1. Electronic databases and applied search strategy.

Database	Search Strategy (November 2019)
PubMed https://www.ncbi.nlm.nih.gov/pubmed	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))
Scopus http://www.scopus.com/	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))
Embase http://www.embase.com/	('Pneumonia, Ventilator-Associated' OR 'Pneumonia, Ventilator Associated'/Exp OR 'Pneumonia, Ventilator Associated' OR 'Ventilator-Pneumonia Associated' OR 'Ventilator Associated Pneumonia'/Exp OR 'Ventilator Associated Pneumonia') AND ('Chlorhexidine'/Exp OR 'Chlorhexidine' OR 'Chlorhexidine Gluconate'/Exp OR 'Chlorhexidine Gluconate')
SciELO www.scielo.org	"Pneumonia Ventilator Associated" AND "Chlorhexidine"
Web of Science http://apps.webofknowledge.com/	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))
LILACS lilacs.bvsalud.org	tw:(tw:("Pneumonia Ventilator Associated" AND "Chlorhexidine") AND (db:("LILACS")))
Cochrane https://www.cochranelibrary.com/	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))
LIVIVO https://www.livivo.de/app	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))
OpenThesis http://www.openthesis.org/	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))

Open Access Thesis and Dissertations https://oatd.org/	((“Pneumonia, Ventilator-Associated” OR “Pneumonia, Ventilator Associated” OR “Ventilator-Pneumonia Associated” OR “Ventilator Associated Pneumonia”) AND (“Chlorhexidine” OR “Chlorhexidine Gluconate”))

Table 2 - Summary of the main characteristics of the eligible studies (all were randomized clinical trials with previous ethical clearance and application of informed consent, with patients receiving mechanical ventilation for more than 48h without pneumonia at baseline).

Author (Year)	Country	Participants	Groups	Sex	Age: Mean (SD)	APACHE, [type]: Mean (SD) [26,27]
						[APACHE III]
Munro *et al, (2009) [11]*	United States	537 patients	Intervention 1: Toothbrushing (three times daily)	M: 28 F: 21	47.9 (17.5)	76.4 (23.3)
			Intervention 2: Toothbrushing (three times daily) + 0.12% CHX (twice daily)	M: 28 F: 20	47.3 (18.8)	76.2 (25.5)
			Control 1: 0.12% CHX/swab (twice daily)	M: 26 F: 18	46.1 (18.2)	80.4 (28.7)
			Control 2: usual care (NR)	M: 37 F: 14	46.8 (16.4)	76.2 (3.3)
Pobo *et al. (2009) [33]*	Spain	147 patients	Intervention: Standard care + toothbrushing (three times daily)	M: 49 F: 25	55.3 (17.9)	[APACHE II] 18.8 (7.1)
			Control: Standard care (gauze containing 20mL of 0.12% CHX applied to teeth, tongue, and the mucosal surface + 10mL of 0.12% CHX digluconate was injected into the oral cavity (three times daily)	M: 46 F: 27	52.6 (17.2)	18.7 (7.3)
Lorente *et al. (2012) [2]*	Spain	436 patients	Intervention: 0.12% CHX -impregnated gauze + toothbrushing of the teeth with 0.12% CHX (three times daily)	M: 146 F: 71	61 (15.6)	[APACHE II] 17.88 (8.84)
			Control: 0.12% CHX -impregnated gauze and oral cavity injection only (three times daily)	M: 145 F: 74	60.4 (16.6)	19.16 (9.88)
Vidal *et a, (2017) [34]*	Brazil	213 patients	Intervention: toothbrushing + 0.12% CHX (twice daily)	M: 51 F: 54	59.4 (14.5)	[APACHE II] 21.9 (7.5)
			Control: swab + 0.12% CHX (twice daily)	M: 54 F: 54	63.2 (14.5)	22.2 (7.7)

SD – Standard Deviation; APACHE – Acute Physiology and Chronic Health assessment; M - male; F- female.

Table 3 - Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for randomized clinical trial studies [28].

Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	Q.11	Q.12	Q.13	% yes/risk
Munro et al. [11]	√	√	√	N/A	U	U	--	√	√	√	√	√	√	69.2% / low risk of bias
Pobo et al. [33]	√	√	√	N/A	U	U	--	√	√	√	√	√	√	69.2% / low risk of bias
Lorente et al. [2]	√	U	√	N/A	U	U	√	√	√	√	√	√	√	69.2% / low risk of bias
Vidal et al. [34]	√	√	√	N/A	√	√	√	√	√	√	√	√	√	92.3% / low risk of bias

Q.1 - Was true randomization used for assignment of participants to treatment groups?; Q.2 - Was allocation to treatment groups concealed?; Q.3 Were treatment groups similar at the baseline?; Q.4 - Were participants blind to treatment assignment?; Q.5 - Were those delivering treatment blind to treatment assignment?; Q.6 - Were outcomes assessors blind to treatment assignment?; Q.7 - Were treatment groups treated identically other than the intervention of interest?; Q.8 - Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?; Q.9 - Were participants analyzed in the groups to which they were randomized?; Q.10 - Were outcomes measured in the same way for treatment groups?; Q.11 - Were outcomes measured in a reliable way?; Q.12 - Was appropriate statistical analysis used?; Q.13 - Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial? / √ - Yes; -- - No; U – Uncertain; N/A – Not applicable.

Table 4. Summary of the outcomes of the eligible studies.

Author	VAP Incidence	Days Ventilated, mean (SD)	Mortality (VAP)	Microbiology
Munro *et al. [11]*	IG1: NR /49 IG2: NR /48 CG1: NR/44 CG2: NR /51	NR	NR	YES
Pobo *et al. [33]*	IG:15/74 CG:18/73	8.9 (5.8) 9.8 (6.1)	NR	YES
Lorente *et al. [2]*	IG: 21/217 CG: 24/219	9.18 (14.13) 9.93(15.39)	NR	YES
Vidal *et al. [34]*	IG:17/105 CG: 28/108	8.7 (5.0) 11.1 (7.6)	NR	YES

IG- intervention group; CG- control group.

Table 5. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Summary of Findings Table for the Outcomes of the Systematic Review and Meta-Analysis [29].

Is toothbrushing combined with the use of 0.12% CHX in patients undergoing mechanical ventilation more effective for preventing VAP than using only CHX?											
Quality Assessment							Summary of Results				Importance
Number of studies	**Study Design**	**Methodological Limitations**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Number of participants**		**Effect**	**General Quality**	
							Intervention	Control	Random RR (95% CI)		
3	Randomised trials	Serious[1]	Not serious	Not serious	Not serious	none	396	401	0.76 (0.55 – 1.06)	⊕⊕⊕ Moderate	Critical

GRADE Working Group grades of evidence

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

[1] There is limitation regarding the blindness of the participants, of those delivering treatment and of outcomes assessors.

Figure 1. Flowchart of the process of literature search and selection adapted from the PRISMA statement.

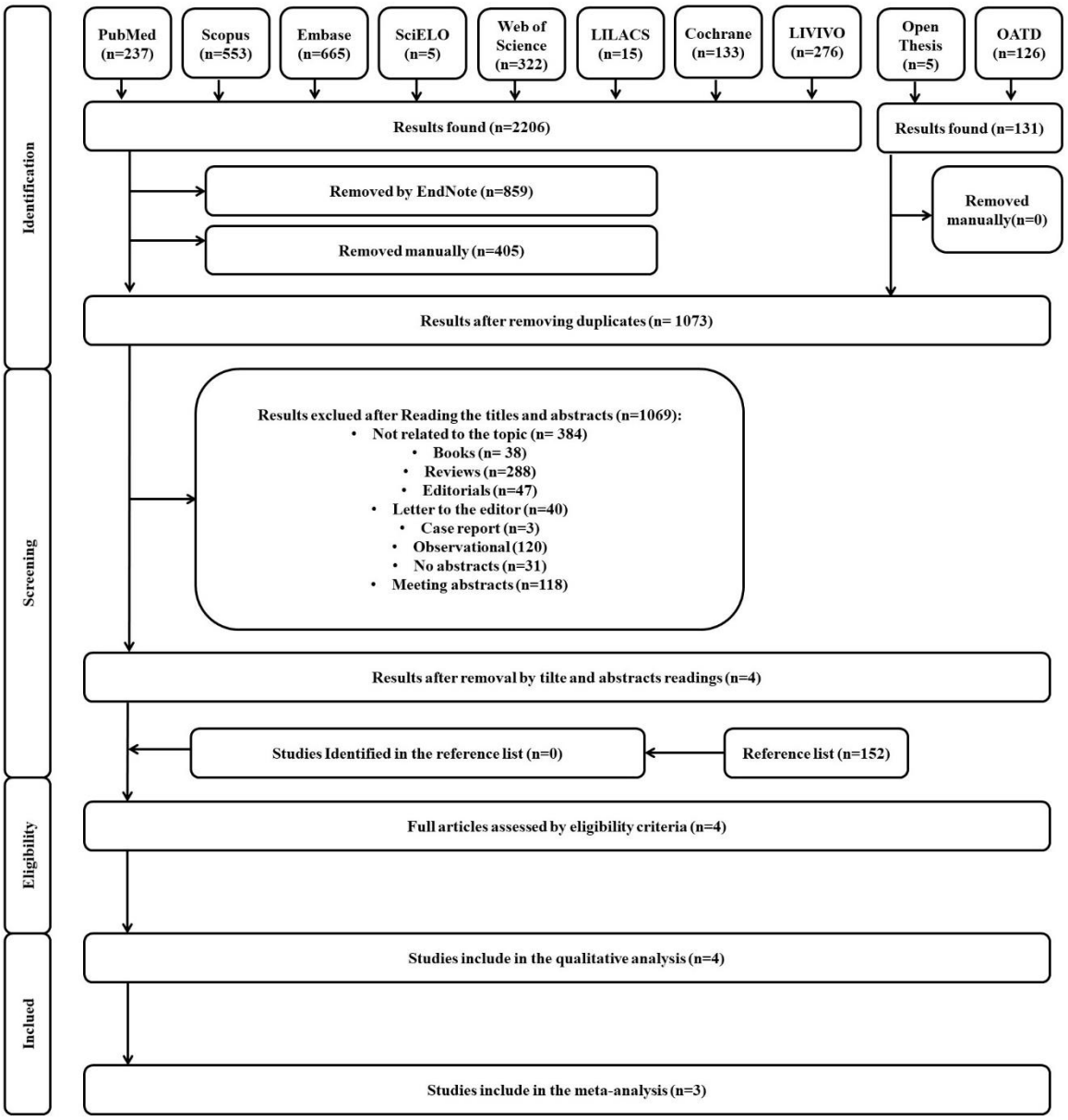
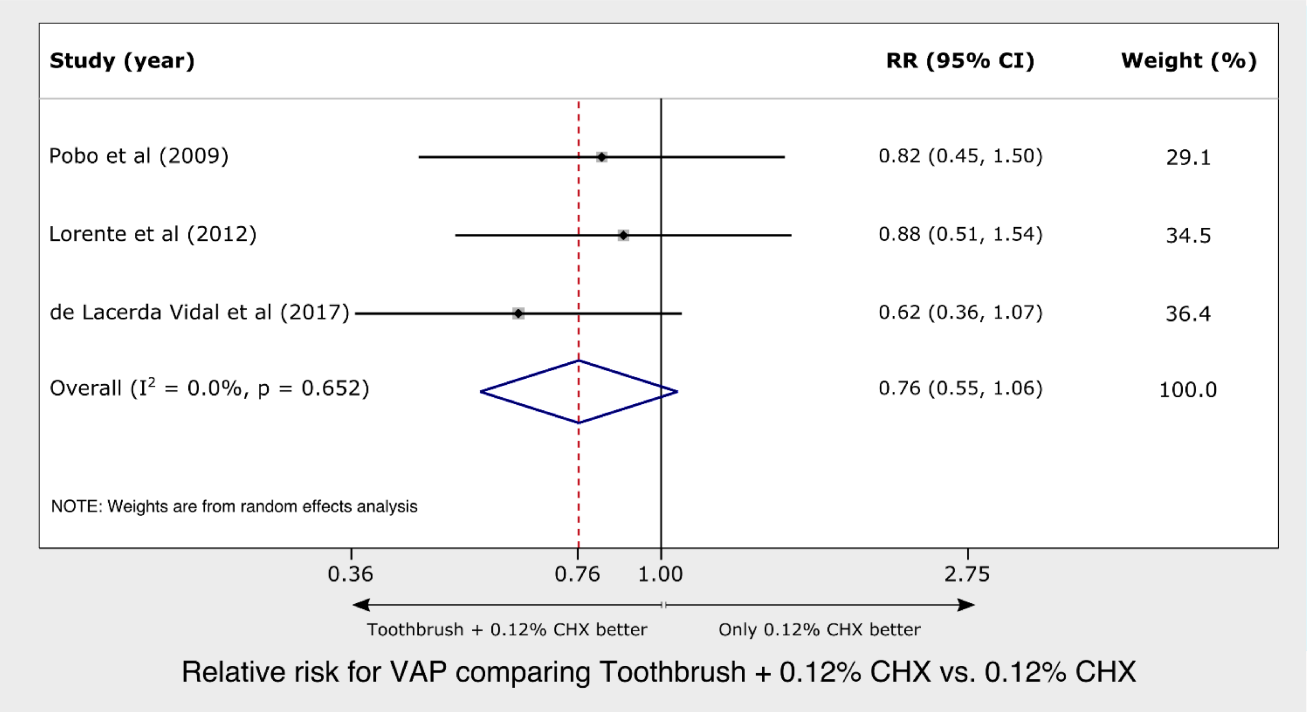


Figure 2. Forest plot comparing groups CHX 0.12% + toothbrushing and CHX 0.12% only.



REFERÊNCIAS

1. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005;171(4):388-416. https://doi.org/10.1164/rccm.200405-644ST

2. Atashi V, Yousefi H, Mahjobipoor H, Bekhradi R, Yazdannik A. Effect of oral care program on prevention of ventilator-associated pneumonia in intensive care unit patients: a randomized controlled trial. Iran J Nurs Midwifery Re. 2018;23:486-490. https://doi.org/10.4103/ijnmr.IJNMR_164_17

3. Berra L, Panigada M, De Marchi L, et al. New approaches for the prevention of airway infection in ventilated patients. Lessons learned from laboratory animal studies at the National Institutes of Health. Minerva Anestesiol. 2003;69(5):342-347.

4. Bouadma L, Sonneville R, Garrouste-Orgeas M, et al. Ventilator-Associated Events: Prevalence, Outcome, and Relationship With Ventilator-Associated Pneumonia. Crit Care Med. 2015;43(9):1798-1806. https://doi.org/10.1097/CCM.0000000000001091

5. Chacko, R., Rajan, A., Lionel, P., Thilagavathi, M., Yadav, B., & Premkumar, J. (2017). Oral decontamination techniques and ventilator-associated pneumonia. British Journal of Nursing, 26(11), 594-599. https://doi.org/10.12968/bjon.2017.26.11.594

6. Conley P, McKinsey D, Graff J, Ramsey AR. Does an oral care protocol reduce VAP in patients with a tracheostomy?. Nursing. 2013;43(7):18-23. https://doi.org/10.1097/01.NURSE.0000428709.81378.7c

7. Cutts S, Talboys R, Paspula C, Prempeh EM, Fanous R, Ail D. Adult respiratory distress syndrome. Ann R Coll Surg Engl. 2017;99(1):12-16. https://doi.org/10.1308/rcsann.2016.0238

8. de Lacerda Vidal CF, Vidal AK, Monteiro JG Jr, et al. Impact of oral hygiene involving toothbrushing versus chlorhexidine in the prevention of ventilator-associated pneumonia: a randomized study [published correction appears in BMC Infect Dis. 2017 Feb 27;17 (1):173]. BMC Infect Dis. 2017;17(1):112. Published 2017 Jan 31. https://doi.org/10.1186/s12879-017-2188-0

9. Ego A, Preiser JC, Vincent JL. Impact of diagnostic criteria on the incidence of ventilator-associated pneumonia. Chest. 2015;147(2):347-355. https://doi.org/10.1378/chest.14-0610

10. El Solh AA, Pietrantoni C, Bhat A, Bhora M, Berbary E. Indicators of potentially drug-resistant bacteria in severe nursing home-acquired pneumonia. Clin Infect Dis. 2004;39(4):474-480. https://doi.org/10.1086/422317

11. Ellepola AN, Joseph BK, Khan ZU. Changes in the cell surface hydrophobicity of oral Candida albicans from smokers, diabetics, asthmatics, and healthy individuals following limited exposure to chlorhexidine gluconate. Med Princ Pract.

12. Grossman RF, Fein A. Evidence-based assessment of diagnostic tests for ventilator-associated pneumonia. Executive summary. Chest. 2000;117(4 Suppl 2):177S-181S. https://doi.org/10.1378/chest.117.4_suppl_2.177S

13. Hirata K, Kurokawa A. Chlorhexidine gluconate ingestion resulting in fatal respiratory distress syndrome. Vet Hum Toxicol. 2002;44(2):89-91.

14. How-to Guide: Prevent Ventilator-Associated Pneumonia. Cambridge, MA: Institute for Healthcare Improvement; 2012. (Available at www.ihi.org).

15. Hugo WB, Longworth AR. Some aspects of the mode of action of chlorhexidine. J Pharm Pharmacol. 1964;16:655-662. https://doi.org/10.1111/j.2042-7158.1964.tb07384.x

16. Hutchins K, Karras G, Erwin J, Sullivan KL. Ventilator-associated pneumonia and oral care: a successful quality improvement project. Am J Infect Control. 2009;37(7):590-597. https://doi.org/10.1016/j.ajic.2008.12.007

17. Jackson L, Owens M. Does oral care with chlorhexidine reduce ventilator-associated pneumonia in mechanically ventilated adults?. Br J Nurs. 2019;28(11):682-689. https://doi.org/10.12968/bjon.2019.28.11.682

18. Kishimoto H, Urade M. Mechanical tooth cleaning before chlorhexidine application. Am J Respir Crit Care Med. 2007;175(4):418. https://doi.org/10.1164/ajrccm.175.4.418a

19. Klompas M. Potential Strategies to Prevent Ventilator-associated Events. Am J Respir Crit Care Med. 2015;192(12):1420-1430. https://doi.org/10.1164/rccm.201506-1161CI

20. Long Y, Mou G, Zuo Y, Lv F, Feng Q, Du J. Effect of modified oral nursing method on the patients with orotracheal intubation. J Nurs Training. 2012;27:2290-2293.

21. Lorente L, Lecuona M, Jiménez A, et al. Ventilator-associated pneumonia with or without toothbrushing: a randomized controlled trial. Eur J Clin

Microbiol Infect Dis. 2012;31(10):2621-2629.
https://doi.org/10.1007/s10096-012-1605-y

22. McCue MK, Palmer GA. Use of Chlorhexidine to Prevent Ventilator-Associated Pneumonia in a Long-term Care Setting: A Retrospective Medical Record Review. J Nurs Care Qual. 2019;34(3):263-268.
https://doi.org/10.1097/NCQ.0000000000000367

23. Melsen WG, Rovers MM, Koeman M, Bonten MJ. Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies. Crit Care Med. 2011;39(12):2736-2742.
https://doi.org/10.1097/CCM.0b013e3182281f33

24. Metheny NA, Stewart BJ, McClave SA. Relationship between feeding tube site and respiratory outcomes. JPEN J Parenter Enteral Nutr. 2011;35(3):346-355.
https://doi.org/10.1177/0148607110377096

25. Mori H, Hirasawa H, Oda S, Shiga H, Matsuda K, Nakamura M. Oral care reduces incidence of ventilator-associated pneumonia in ICU populations. Intensive Care Med. 2006;32(2):230-236.
https://doi.org/10.1007/s00134-005-0014-4

26. Munro CL, Grap MJ, Elswick RK Jr, McKinney J, Sessler CN, Hummel RS 3rd. Oral health status and development of ventilator-associated pneumonia: a descriptive study. Am J Crit Care. 2006;15(5):453-460.

27. Okgün Alcan A, Demir Korkmaz F, Uyar M. Prevention of ventilator-associated pneumonia: Use of the care bundle approach. Am J Infect Control. 2016;44(10):e173-e176.
https://doi.org/10.1016/j.ajic.2016.04.237

28. Orito K, Hashida M, Hirata K, Kurokawa A, Shirai M, Akahori F. Effects of single intratracheal exposure to chlorhexidine gluconate on the rat lung. Drug Chem Toxicol. 2006;29(1):1-9.
https://doi.org/10.1080/01480540500408416

29. Park DR. The microbiology of ventilator-associated pneumonia. Respir Care. 2005;50(6):742-765.

30. Rebmann T, Greene LR. Preventing ventilator-associated pneumonia: An executive summary of the Association for Professionals in Infection Control and Epidemiology, Inc, Elimination Guide. Am J Infect Control. 2010;38(8):647-649.
https://doi.org/10.1016/j.ajic.2010.08.004

31. Septimus JE, Schweizerc ML. Decolonization in Prevention of Health Care-Associated Infections Clinical Microbiology Reviews. April 2016 Volume 29 Number 2.
https://doi.org/10.1128/CMR.00049-15

32. Shinn, J. A. Decreasing the Risk of Ventilator-Associated Pneumonia: The Impact of Nursing Care. Progress in Cardiovascular Nursing, 2009; 19(3), 85-86.
https://doi.org/10.1111/j.0889-7204.2004.02904.x

33. Shrada.B.Kumar. Chlorhexidine Mouthwash- A Review. J. Pharm. Sci. & Res. Vol. 9(9), 2017, 1450-1452.

34. Soh KL, Soh KG, Japar S, Raman RA, Davidson PM. A cross-sectional study on nurses' oral care practice for mechanically ventilated patients in Malaysia. J Clin Nurs. 2011;20(5-6):733-742.
https://doi.org/10.1111/j.1365-2702.2010.03579.x

35. Sona CS, Zack JE, Schallom ME, et al. The impact of a simple, low-cost oral care protocol on ventilator-associated pneumonia rates in a surgical intensive

care unit. J Intensive Care Med. 2009;24(1):54-62
https://doi.org/10.1177/0885066608326972

36. Spalding MC, Cripps MW, Minshall CT. Ventilator-Associated Pneumonia: New Definitions. Crit Care Clin. 2017;33(2):277-292.
https://doi.org/10.1016/j.ccc.2016.12.009

37. Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V. Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. Infect Control Hosp Epidemiol. 2008;29(2):131-136.
https://doi.org/10.1086/526438

38. ten Cate JM. Biofilms, a new approach to the microbiology of dental plaque. Odontology. 2006;94(1):1-9.
https://doi.org/10.1007/s10266-006-0063-3

39. Vandana Kalwaje E, Rello J. Management of ventilator-associated pneumonia: Need for a personalized approach. Expert Rev Anti Infect Ther. 2018;16(8):641-653.
https://doi.org/10.1080/14787210.2018.1500899

40. Wei HP, Yang K. Effects of different oral care scrubs on ventilator-associated pneumonia prevention for machinery ventilates patient: A protocol for systematic review, evidence mapping, and network meta-analysis. Medicine (Baltimore). 2019;98(12): e14923.
https://doi.org/10.1097/MD.0000000000014923

41. Wu D, Wu C, Zhang S, Zhong Y. Risk Factors of Ventilator-Associated Pneumonia in Critically III Patients. Front Pharmacol. 2019;10:482. Published 2019 May 9.
https://doi.org/10.3389/fphar.2019.00482

42. Xie X, Lyu J, Hussain T, Li M. Drug Prevention and Control of Ventilator-Associated Pneumonia. Front Pharmacol. 2019;10:298. Published 2019 Mar 28. https://doi.org/10.3389/fphar.2019.00298

43. Xue Y, Zhang S, Yang Y, et al. Acute pulmonary toxic effects of chlorhexidine (CHX) following an intratracheal instillation in rats. Hum Exp Toxicol. 2011;30(11):1795-1803. https://doi.org/10.1177/0960327111400104