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Efetividade dos Probióticos na Prevenção de Infecções em Idosos: Revisão Sistemática e Metanálise

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutor em Saúde Coletiva.

Orientador: Prof. Dr. Paulo José Fortes Villas Boas.

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Botucatu, 23 de Maio de 2017.

À minha família e aos colegas, amigos
e professores da Faculdade de Medicina de Botucatu.

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RESUMO

WACHHOLZ, P.A. **Efetividade dos probióticos na prevenção de infecções em idosos: revisão sistemática e Metanálise**. 2017. 169 f. Tese (Doutorado). Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, 2017.

O impacto das doenças infecciosas na pessoa idosa extrapola os domínios clínico, econômico e social: à redução da sobrevida somam-se o elevado risco de declínio funcional, maior demanda por cuidados e significativa piora na qualidade de vida. Intervenções que possam prevenir infecções em idosos ou reduzir seu impacto podem agregar valor à estratégias bem embasadas, como a vacinação, nutrição e medidas de higiene e proteção à saúde. Os probióticos são microrganismos vivos que, quando consumidos em quantidades suficientes, conferem benefícios à saúde do hospedeiro. Evidências sugerem que o consumo de probióticos possa associar-se à redução na duração média e frequência dos episódios de infecções de vias aéreas superiores e diarreias infecciosas, porém os resultados de ensaios clínicos realizados com idosos são controversos. Esta revisão sistemática e metanálise objetiva avaliar a efetividade e segurança dos probióticos na prevenção de infecções em idosos. Utilizando estratégias sistematizadas, buscou-se por ensaios clínicos disponíveis na literatura até 30 de Dezembro de 2016, que tivessem incluído preferencialmente pessoas com mais de 65 anos, comparando o uso de probióticos com placebo. Dois autores acessaram de modo independente a elegibilidade e qualidade dos estudos, e extraíram os dados utilizando os procedimentos metodológicos recomendados pela Colaboração Cochrane. Os principais desfechos de eficácia incluíram a prevenção de infecções, a qualidade de vida, a mortalidade e a duração média por episódio de infecção, enquanto os desfechos de segurança incluíram os eventos adversos. Os resultados foram expressos como razões de risco, diferença média ou diferença média padronizada, conforme apropriado, com intervalos de confiança de 95%, utilizando metanálises de efeitos aleatórios, quando pelo menos três estimativas estiverem disponíveis por desfecho. Quando identificou-se substancial heterogeneidade, foram investigadas as causas potenciais, incluindo

análises de subgrupos. Para avaliar a qualidade da evidência para cada um dos desfechos de interesse utilizamos a ferramenta GRADE. Resultados principais: A busca resultou em 5.036 citações; excluídas as duplicatas e aplicados os critérios de elegibilidade, 15 estudos foram incluídos. Estes avaliaram 5.916 participantes, com uma idade média de 75,21 anos, durante uma mediana de seguimento de 319 dias (204,75—631,50). O efeito dos probióticos na ocorrência de infecções não diferiu daquele observado para o placebo (13 estudos, 5.820 participantes: RR 0,90, IC95% 0,76—1,08, I^2=24%, moderada qualidade da evidência). Não foram observadas diferenças significativas nas estimativas de efeito quando analisados os estudos que incluíram apenas pessoas idosas, bem como nas análise de subgrupos ou de sensibilidade. Os efeitos do consumo de probióticos na ocorrência de eventos adversos e mortalidade geral não foram estatisticamente diferentes daqueles observados para o placebo (RR 1,01, IC95%, 0,91—1,12; I^2=0%, moderada qualidade da evidência e RR 1,09, IC95% 0,70—1,72; I^2=5%, muito baixa qualidade da evidência, respectivamente). Por fim, a duração média dos episódios de infecção não diferiu entre os idosos que consumiram probióticos quando comparados aos que usaram placebo (sete estudos, 1.406 participantes, MD -0,35, IC95% -1,57—0,87, I^2=86%, baixa qualidade da evidência). Análises de subgrupo não foram capazes de reduzir a heterogeneidade deste desfecho, e análise de sensibilidade não modificaram as estimativas de efeito. Conclusões: A utilização de probióticos com o objetivo de prevenir infecções ou reduzir a duração média de seus episódios não parece diferir do placebo nesta população.

PROTOCOLO PROSPERO(CRD42014013707)

SUPORTE FINANCEIRO: FAPESP Protocolo 2015/03349-0.

Palavras chave: idoso; pessoa idosa; população idosa; idoso de 80 anos ou mais; octogenários; nonagenários; centenários; probióticos; infecções; prevenção de infecções; revisão sistemática; metanálise.

Abstract

WACHHOLZ, P.A. **Effectiveness of probiotics on the prevention of infection in the elderly: Systematic review and meta-analysis**. 2017. 169 p. Thesis (Doctorate degree). Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, 2017.

The burden of infectious diseases on the elderly goes beyond the clinical, economic and social domains: one can add to the reduction on survival higher risk of functional decline, greater demand for care and significant worsening on quality of life. Interventions that could prevent infections in the elderly, or reduce their impact, can add value to well-founded strategies such as vaccination, nutrition, hygiene and health protection measures. Probiotics are living microorganisms that, when consumed in sufficient amounts, confer benefits to the health of the host. Evidence suggests that probiotic consumption may be associated with a reduction in the mean duration and occurrence of episodes of upper respiratory infections and infectious diarrhea, but the results of clinical trials conducted with older patients are controversial. This systematic review and meta-analysis aimed to evaluate the effectiveness and safety of probiotics in the prevention of infections in the elderly. Using systematized strategies, we searched for clinical trials available in the literature up to December 30, 2016, which had preferably included people over 65 years of age, comparing the use of probiotics with placebo. Two authors independently assessed the eligibility and quality of the studies, and extracted the data using the methodological procedures recommended by the Cochrane Collaboration. The main efficacy outcomes included infection prevention, quality of life, and mean duration per episode of infection, while safety outcomes included adverse events and mortality. The results were expressed as risk ratios, mean difference or standardized mean difference, as appropriate, with 95% confidence intervals using random effects meta-analysis, pooled where more than three estimates were available. When substantial heterogeneity was identified, potential causes were investigated, including subgroup analyzes. We used the GRADE tool to evaluate the quality of the evidence for each of the outcomes of interest. Main results: The search resulted in 5,036 citations. Excluding duplicates and applying the eligibility criteria, 15 studies were included.

They evaluated 5,916 participants, with a mean age of 75.21 years, during a median follow-up of 319 days (204,75-631,50). The effect of probiotics on the occurrence of infections did not differ from that observed for placebo (13 studies, 5,820 participants: RR 0.90, 95% CI 0.76-1.08, I2 = 24%, moderate quality of evidence). No significant differences in effect estimates were observed when analyzing studies that included only elderly people, as well as in subgroup or sensitivity analyzes. The effects of probiotic consumption on the occurrence of adverse events and overall mortality were not statistically different from those observed for placebo (RR 1.01, 95% CI, 0.91-1.12, I2 = 0%, moderate quality of evidence, and RR 1.09, 95% CI 0.70-1.72, I2 = 5%, very low quality of evidence, respectively). Finally, the mean duration of infection episodes did not differ between the elderly who consumed probiotics compared to those who used placebo (seven studies, 1,406 participants, MD -0.35, 95% CI -1.57-0.87, I2 = 86%, poor quality of evidence). The subgroup analyzes were not able to reduce the heterogeneity of these outcomes; sensitivity analyzes did not modified the results. CONCLUSIONS: The use of probiotics did not appear to prevent infections or reduce the average duration of their episodes whean compared to placebo in older person.

PROSPERO(CRD42014013707)

Financial Support: Grant # 2015/03349-0 São Paulo Research Foundation (FAPESP).

Key-words: aged; elderly; aged, 80 and over; oldest old; octogenarian; nonagenarians; centenarians; probiotics; infections; infection prevention; systematic review; meta-analysis.

Lista de abreviaturas e siglas

ATS	Avaliação de Tecnologia em Saúde
CCRBt	*"The Cochrane Collaboration Risk of Bias tool*"
CIDRG	"Cochrane Infectious Disease Review Group"
ECR	Ensaio Clínico Randomizado
EPHPP-QAt	"*Effective Public Health Practice Project Quality Assessment tool*"
GRADE	*"Grading of Recommendations Assessment, Development and Evaluation"*
IC	Intervalo de confiança
ITT	*"Intention-to-treat"*
MBE	Medicina Baseada em Evidência
MeSH	*"Medical Subject Headings"*
NK	*"Natural Killer"*
OMS	Organização Mundial de Saúde
OPAS	Organização Pan-Americana de Saúde
PAC	Pneumonia adquirida na comunidade
PBE	Prática Baseada em Evidência
PRISMA	*"Preferred Reporting Items for Systematic Reviews and Meta-Analyses*"
PROSPERO	"*International Prospective Register Of Sistematic Reviews*"
RR	Razão de risco
RS	Revisão Sistemática
SCBE	Saúde Coletiva Baseada em Evidências
SCIE	Saúde Coletiva Informada por Evidências
SUS	Sistema Único de Saúde
UE	União Européia
UUE	Unidade de Urgência e Emergência

Sumário

INTRODUÇÃO

1. Introdução

O número de pessoas com idade igual ou superior a sessenta anos no mundo era de aproximadamente 841 milhões de pessoas em 2013, quase quatro vezes maior que o percentual de pessoas idosas em 1950; estima-se que, até 2050, esse montante possa ultrapassar dois bilhões de pessoas.[1,2] O impacto da transição epidemiológica na carga global de doenças aumentou de modo significativo a demanda por atenção e assistência junto aos sistemas públicos e privados de saúde, principalmente pelo aumento na prevalência das condições crônicas não transmissíveis.

Apesar das reduções importantes nas taxas de mortalidade por causas infecciosas desde o início do século XX,[3] as doenças infecciosas persistem como um importante problema de saúde pública no segmento mais longevo da população: quase 21 milhões de hospitalizações relacionadas a infecções foram registradas em idosos norte-americanos entre 1990 e 2002.[4] Neste mesmo país, em 2012, as visitas de idosos a unidades de urgências por causas infecciosas ultrapassaram 3 milhões de atendimentos (7,231 por 100.000 pessoas com 65 anos ou mais), exibindo um impacto superior ao observado para os atendimentos por insuficiência cardíaca congestiva e infarto do miocárdio combinados.[3] Nos subgrupos de idosos frágeis e institucionalizados o impacto, a gravidade e frequência das infecções é ainda maior: aproximadamente 70% das causas de óbito em instituições de longa permanência podem ser atribuídas a causas infecciosas.[5]

As infecções na velhice são, indubitavelmente, mais freqüentes, mais graves e associadas a maior risco de mortalidade e declínio funcional do que em adultos jovens.[6] A maior susceptibilidade dos idosos às infecções está comprovadamente associada a imunosenescência, um processo inerente ao envelhecimento fisiológico,[7–9] que inclui alterações na distribuição e função das células envolvidas nas respostas inatas e adaptativas do sistema imune.[7]

Diversas estratégias e intervenções têm sido descritas com o objetivo de atenuar o impacto da imunosenescência;[10–13] em sua grande maioria, porém, ainda são insuficientes ou ausentes as evidências de eficácia destas em desfechos clinicamente significativos.

Em mais de 15 anos de prática clínica em geriatria tivemos a oportunidade de assistir a pacientes idosos em todos os níveis de atenção e ambientes de convivência, tanto em caráter público quanto privado. De fato, a preocupação com o risco de infecções e seus desfechos (principalmente em quadros graves e associados a hospitalização) sempre permeou nossa prática cotidiana. Em muitas ocasiões, pacientes e família nos

questionavam quais intervenções poderiam ser benéficas para “melhorar a imunidade”, ou para reduzir o risco de novos episódios de infecções. E, à exceção das recomendações pertinentes às imunizações, havia muito pouca evidência consistente que nos permitisse recomendar qualquer intervenção profilática neste segmento etário.

De fato, a qualidade das evidências diretas disponíveis para intervenções em pacientes idosos ainda é bastante limitada pela insuficiente inclusão deste grupo etário em ensaios clínicos randomizados (ECR); infelizmente, frequentemente empregamos intervenções em geriatria “extrapolando” os achados de ECR delineados para segmentos etários não envelhecidos.

Dentre as intervenções que já foram analisadas em outros segmentos etários com o objetivo de reduzir a ocorrência de episódios de infecção destaca-se o consumo de probióticos. Do ponto de vista teórico, os probióticos parecem reunir algumas vantagens interessantes quando comparados com outras intervenções, particularmente relacionadas à boa tolerabilidade e ao perfil favorável de segurança clínica, com reduzida prevalência de eventos adversos.

Diversos ECR e Revisões Sistemáticas (RS) consistentemente encontraram evidência de redução na incidência, duração e gravidade de infecções respiratórias e do trato gastrointestinal em lactentes e crianças.[14–19] Porém, ECR conduzidos exclusivamente com populações idosas demonstraram resultados controversos: enquanto alguns sugeriam redução na duração, gravidade ou ocorrência de infecção,[14–18] outros não encontraram os mesmos resultados.[19–21]

Realizamos, então, uma busca na literatura com vistas a identificar se já havia alguma RS publicada sobre este tema (ou um protocolo de RS em andamento) junto às principais bases de dados e plataformas de registro de RS (Cochrane Library, PROSPERO e Joanna Briggs). Como estas buscas não identificaram publicações sobre o tema, propusemos-nos a elaborar esta RS. Definimos, pois, nossa pergunta de pesquisa: “*O consumo de probióticos é efetivo e seguro na prevenção de infecções em idosos?”*

Didaticamente, estruturamos a pergunta de pesquisa na estratégia PICO, conforme ilustrado no quaro 1

Quadro 1. Apresentação dos componentes da estratégia “P.I.C.O.” para a questão de pesquisa desta revisão sistemática. Botucatu, 2017.

P	**Idosos (pessoas com 65 anos ou mais)**
I	**Consumo de probióticos**
C	**Placebo**
O	**Prevenção de infecções**

Assumimos como principais hipóteses de pesquisa que:

a) *O consumo de probióticos por idosos, comparado com placebo, previne a ocorrência de infecções, ou seja, reduz a ocorrência destes episódios;*
b) *O consumo de probióticos por idosos, comparado com placebo, reduz a duração dos episódios de infecção;*
c) *O perfil e frequência de eventos adversos relacionados ao consumo de probióticos por idosos não difere significativamente do placebo.*

Tendo em vista a paucidade de informações, publicações e evidências envolvendo o consumo de probióticos e a prevenção de infecções em idosos, bem como a relevância do tema para a prática clínica, decidimos apresentar o conteúdo desta tese no formato de artigos científicos, primando pela disseminação do conhecimento construído neste processo. Deste modo, enquanto realizávamos as primeiras buscas na literatura, identificamos a possibilidade de publicação de duas cartas ao editor, em periódicos nas áreas de nutrição e geriatria (Anexos 1 e 2).

Durante a elaboração e inscrição do protocolo desta revisão sistemática junto a *International Prospective Register of Systematic Reviews - PROSPERO [Anexo 3],* recebemos um convite para redigir um capítulo de livro sobre o tema para a segunda edição da obra “*Probiotics, prebiotics and synbiotics*”, publicado pela Editora *Academic Press*, sob a chancela da Editora Elsevier (Publicação 1).

Pouco tempo depois, publicamos o protocolo desta revisão sistemática no periódico *Nutrition Bulletin* (*Wiley Library*), veículo científico oficial da *British Nutrition Foundation (Publicação 2)*.

Durante a fase final de elaboração desta tese, identificamos o potencial para publicação de parte do material de nossa revisão de literatura que versa sobre a prática baseada em evidências no campo da Saúde Coletiva, manuscrito que foi submetido para

publicação neste ano de 2017. Por fim, optamos por apresentar também os resultados, discussão e conclusões desta revisão sistemática e metanálise no formato artigo, na língua inglesa, com vistas a pronta submissão deste.

REVISÃO DE LITERATURA

2. Revisão da literatura

2.1 Capítulo 1: Da prática baseada em evidências para a Saúde Coletiva Informada por evidências: percursos, avanços e obstáculos

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Prezado(a) Dr. Wacholz:

Agradecemos a submissão do seu manuscrito intitulado "DA PRÁTICA BASEADA EM EVIDÊNCIAS PARA A SAÚDE COLETIVA INFORMADA POR EVIDÊNCIAS: PERCURSOS, AVANÇOS E OBSTÁCULOS" para Saúde e Sociedade.

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DA PRÁTICA BASEADA EM EVIDÊNCIAS PARA A SAÚDE COLETIVA INFORMADA POR EVIDÊNCIAS: PERCURSOS, AVANÇOS E OBSTÁCULOS

FROM EVIDENCE-BASED PRACTICE TO EVIDENCE-INFORMED PUBLIC HEALTH: PATHWAYS, ADVANCES AND OBSTACLES.

Resumo

A adoção de evidências científicas no campo da Saúde Coletiva tem a finalidade de garantir otimização de recursos e maior efetividade nas ações de promoção, prevenção e atenção à saúde. Apesar do reconhecimento da importância do conhecimento científico baseado em evidências para o desenvolvimento de políticas públicas, o uso dessa ferramenta para subsidiar a tomada de decisão ainda é incipiente. O presente estudo teve como objetivo descrever a trajetória do uso do conhecimento baseado em evidências na prática da Saúde Coletiva. Foi realizado um levantamento bibliográfico não sistemático em periódicos nacionais e internacionais; os resultados são apresentados em três eixos norteadores: as evidências e a prática em saúde; as evidências em saúde Coletiva e; os avanços e desafios da Saúde Coletiva Informada pelas Evidências. O estudo permitiu concluir que as decisões na Saúde Coletiva são mais complexas do que as decisões clínicas individuais, pois envolvem avaliações de impactos orçamentários e políticos, reforçando a relevância da adoção de práticas informadas pelas evidências neste campo.

Palavras-chave: Saúde Pública; Medicina baseada em evidências; Tomada de decisões

Abstract

The adoption of scientific evidence in the field of Public Health has the purpose of guaranteeing optimization of resources and greater effectiveness in the actions of promotion, prevention and health care. Despite the recognition of the importance of evidence-based scientific knowledge for the development of public policies, the use of this tool to subsidize decision-making is still incipient. The present study aimed to describe the trajectory of the use of evidence in the practice of Public Health. A non - systematic bibliographic review was carried out in national and international journals. The results are presented in three guiding axes: the evidences and the practice in health; The evidence in Public Health and; The advances and challenges of Public Health Informed by the Evidences. The study allowed us to conclude that decisions in Public Health are more complex than individual clinical decisions, since they involve evaluations of budgetary and political impacts, reinforcing the relevance of the adoption of practices informed by the evidences in this field.

Key-words: Public health; Evidence-based medicine; decision making

INTRODUÇÃO

Um dos grandes desafios globais para as políticas de saúde é a promoção da utilização de evidências científicas para sua formulação e implementação. Em países como o Brasil, onde as políticas públicas de saúde sofrem fortes influências de fatores socioeconômicos e da limitação orçamentária, este desafio é ainda maior (Brasil, 2014; Dias et al., 2015). A adoção de evidências científicas na prática da Saúde Coletiva tem o objetivo de garantir otimização de recursos e maior efetividade nas ações de promoção, prevenção e atenção à saúde (Brasil, 2014).

A despeito do uso de evidências na Saúde Coletiva ser possível e desejável, a transposição dos conceitos “baseados em evidências” para este campo não é direta. As complexas ações envolvidas na determinação social das doenças e no processo de trabalho em saúde, típicos da Saúde Coletiva, diferem substancialmente da simplificação usualmente envolvida nas práticas individuais de saúde.

Do mesmo modo, o uso das evidências pelos gestores de saúde com vistas a subsidiar a tomada de decisão ainda é elementar em nosso meio (Dias et al., 2015). Neste contexto, após realização de levantamento bibliográfico não sistematizado em periódicos nacionais e internacionais, verificou-se a importância da descrição e caracterização da trajetória de utilização das evidências científicas no campo da Saúde Coletiva. Este manuscrito tem como objetivo analisar a evolução destes conceitos e o emprego das evidências no âmbito da Saúde Coletiva, bem como discutir as principais dificuldades e iniciativas que possam permitir aproximações entre estes. Os principais achados são apresentados em três eixos norteadores: *as evidências e a prática em saúde*; *as evidências em saúde Coletiva* e; *os avanços e desafios da Saúde Coletiva Informada pelas Evidências*

As evidências e a prática em saúde

O conceito de "evidência" surge pela primeira vez por meio de René Descartes, na primeira metade do século XVII, a partir da obra "*Discours de la méthode*". Nela, o filósofo transmite sua preocupação por encontrar um método para conhecer a verdade que, ao ser utilizado, permitisse aceitar como verdadeiro apenas aquilo que fosse evidente (Socorro, 2006).

Contemporaneamente, o termo "evidências científicas" tem sido empregado como o conjunto de informações utilizadas para confirmar ou negar uma teoria ou hipótese científica. Deste modo, evidências são produzidas quando estudos e pesquisas são conduzidos com a intenção de esclarecer a relação, efeito ou causalidade entre duas ou mais variáveis, condições ou intervenções.

As primeiras referências ao termo "Medicina Baseada em Evidências" (MBE) foram publicadas por Guyatt e Sackett (Evidence-based medicine working group, 1992; Sackett & Rosenberg, 1995), na época eminentes epidemiologistas clínicos na Universidade de *McMaster* (Canadá). Posteriormente, seu grupo e discípulos estruturaram e fomentaram a disseminação do conceito, atraindo protagonistas principalmente na América do Norte (Jenicek, 1997). A MBE surge, então, como um movimento científico que se traduz pela recomendação de que prática da medicina deva acontecer em um contexto aonde a experiência profissional integre-se com a capacidade de analisar criticamente a informação científica disponível, e aplicá-la de forma racional, em parceria com o(s) sujeito(s) envolvido(s), de forma a melhorar a qualidade da assistência (Lopes, 2000).

Sem pretensões niilistas, a MBE caracterizou-se por recomendar o uso judicioso e explícito da melhor evidência disponível na tomada de decisões, sempre com foco no cuidado individual com a saúde (Sackett & Rosenberg, 1995; Jenicek, 1997; Glasziou & Longbottom, 1999). Ainda que distâncias importantes viessem a ser reconhecidas entre as evidências geradas pelas análises epidemiológicas e a prática em saúde, a MBE forneceu importantes subsídios que permitiram avanços na validação das informações, na análise da eficácia e efetividade das intervenções em saúde, bem como, presumivelmente, da prática médica (Shelton, 2014).

Gradualmente, a MBE foi sendo incorporada, apropriada e adaptada por diferentes contextos, cenários e movimentos, e seu construto evoluiu paulatinamente para conceito mais amplo de "Prática Baseada em Evidências" (PBE) (Ammerman, Smith & Calancie, 2014). A evidência, *per se*, não era capaz de direcionar ou modificar as decisões em saúde; ou seja, a PBE partia do pressuposto de que os tomadores de decisão deveriam levar em conta as necessidades e valores do sujeito (e também das populações), bem como a disponibilidade de recursos para sua implementação (Gray, 1997).

Mediante o incomensurável aumento no volume de informações e evidências potencialmente disponíveis para o uso na prática profissional em nível individual, bem

como para a estruturação das políticas públicas e/ou decisões operacionais em saúde, a PBE destacava-se como uma alternativa pertinente e atraente (Gray, 1997; Jenicek, 1997). Ao disponibilizar estratégias para a seleção das melhores evidências disponíveis, e recomendar o uso eficaz destas informações, esperava-se fornecer condições para que os gestores públicos pudessem adotar "melhores práticas" nas tomadas de decisão (Mowat, 2007; Cediel-Becerra & Krause, 2013).

Neste contexto, é fundamental perceber que "criar evidências" e "criar ações em saúde" são processos absolutamente distintos, sendo as pesquisas científicas (e todas as formas de produzir evidências) consideradas atos de *produção do conhecimento*; por outro lado, a criação de ações em saúde (por exemplo, *tomada de decisão*), que pode utilizar (ou não) as evidências produzidas e disponíveis, constituem-se fundamentalmente em atos políticos ou resultantes das relações sociais de poder (Brasil, 2015).

A apropriação e o emprego das evidências científicas passaram a ser uma conduta recomendada e preconizada pela Organização Mundial de Saúde (OMS) (Tandon et al., 2001), com vistas a melhorar o desempenho dos sistemas de saúde entre seus membros; do mesmo modo, a Organização Pan-Americana de Saúde (OPAS) incluiu a aplicação de evidências científicas entre as linhas de ação recomendadas para o fortalecimento da capacidade institucional na agenda de saúde para as Américas (2008-2017) (Pan american health organization, 2007).

A transferência do conceito "baseado em evidência" da prática clínica para o campo de conhecimentos e práticas da Saúde Coletiva, porém, não tem sido tão direta (Cediel-Becerra & Krause, 2013). Nesse contexto, saúde coletiva significa “um esforço organizado da sociedade, principalmente suas instituições públicas, para melhorar, promover, proteger e restaurar a saúde da população mediante ações coletivas” (Pan american health organization, 2003).

As evidências parecem sim condições necessárias, mas não suficientes para responder às perguntas e necessidades da coletividade (Buendía-Rodríguez & Sánchez-Villamil, 2006; Brasil, 2015). Além de saber se uma intervenção funcionará (eficácia), mostrou-se ímpar neste campo indagar se ela funcionaria no "mundo real" (efetividade), a que custo (eficiência), qual seria a distribuição dos riscos e benefícios, se sua implementação seria aceitável e adequada (sem riscos), ou se a capacidade do sistema de

incorporar esta intervenção permitiria sua disponibilização a todos (equidade) (Mowat, 2007).

Enquanto a MBE e a PBE direcionam seu foco principal na prática individual, a Saúde Coletiva toma como objeto as necessidades sociais de saúde voltadas a coletividade, "*concebendo as ações de atenção à saúde como práticas técnicas e sociais*" (Souza, 2014). Particularmente em domínios complexos como a promoção da saúde, aspectos fundamentais como a "aceitabilidade", a adesão e a importância outorgada por uma população à determinada intervenção mostram-se tão ou mais importantes que a qualidade metodológica de uma evidência, ou sua consistência e corroboração (Victora, Habicht & Bryce, 2004; Socorro, 2006).

As evidências no contexto da Saúde Coletiva

No campo e prática da Saúde Coletiva, o construto "Saúde Coletiva baseada em evidências" (SCBE) passou a ser incorporado como um dos instrumentos empregados no ciclo da produção de evidências e respostas necessárias à tomada de decisões (Brasil , 2015).

A SCBE fundamenta-se na integração e uso consciente e crítico da melhor evidência corrente disponível para a tomada de decisão sobre a atenção à comunidade e às populações, nos campos da proteção, prevenção de doenças e promoção da saúde (Duarte, 2003; Cediel-Becerra & Krause, 2013; Brasil, 2015).

De fato, para que melhores decisões sobre intervenções em saúde possam ser adotadas, é fundamental que exista um processo transparente e reprodutível, que considere, por exemplo, se uma intervenção é suficientemente incapaz de causar prejuízo mensurável; geralmente estas informações são provenientes de evidências geradas por estudos observacionais, e quando possível, avaliadas em nível experimental (por meio de ensaios clínicos randomizados - ECR), para que depois possam ser estimadas análises de custo-efetividade (Threlfall et al., 2015).

Inseridas na complexidade contextual da prática em Saúde Coletiva, no entanto, as evidências devem ser consideradas não como uma resposta definitiva para problemas específicos, mas sim como um elemento adicional a ser incorporado na argumentação e debate que norteiam a tomada de decisões, transcendendo o campo epidemiológico e

interagindo com as dimensões social, cultural e político-econômica (Duarte, 2003; Brasil, 2015).

Conforme assinala Vidal (Vidal, 2016), considerando que as intervenções em Saúde Coletiva estão sujeitas "*a um grau de complexidade superior ao das pesquisas clínicas usuais*", é importante perceber algumas diferenças fundamentais entre a PBE e a SCBE:

a) a unidade de análise geralmente é populacional, e não individual;

b) intervenções multicomponentes complexas são frequentemente mais difíceis de serem descritas e caracterizadas do que intervenções simples (como o consumo de um medicamento específico em um ambiente controlado de pesquisa);

c) nem sempre os delineamentos aleatorizados e controlados são possíveis em Saúde Coletiva.

As decisões em saúde coletiva tendem a ser mais complexas do que as decisões clínicas, pois envolvem a avaliação do provável impacto das políticas e outras intervenções complexas, em geral de longo prazo (Mowat & Hockin, 2002). A tomada de decisão em saúde coletiva não é um evento único, mas um processo difuso com várias etapas espalhadas ao longo do tempo, sem uma relação clara e previsível entre elas (Lonas, 2002).

Apesar da SCBE ser tanto possível quanto desejável, o percurso necessário para que uma intervenção testada sobre condições controladas seja reprodutível sob condições rotineiras é muito mais complexo (Victora, Habitch & Bryce, 2004). Pressupostos de probabilidade (baseados estritamente nos resultados de ECR) devem ser complementados por confirmações de plausibilidade (derivadas de delineamentos observacionais com grupos de comparação) e de adequação (efeitos ou tendências no comportamento de indicadores, sugerindo a efetividade da intervenção) (Victora, Habitch & Bryce, 2004; Buendía-Rodríguez & Sánchez-Villamil, 2006).

Nenhum método de pesquisa é capaz de, individualmente, oferecer respostas suficientes para a imediata aplicação de uma evidência no campo da coletividade (Shelton, 2014; Hatt et a., 2015; Threlfall et al., 2015). Diferentemente da aplicação individual da informação advinda de ECR, a Saúde Coletiva deve ser capaz de responder a demanda de populações extremamente heterogêneas, incluindo complexidade e variabilidade que

usualmente não são generalizáveis a partir do resultado de estudos com foco apenas na eficácia de uma intervenção (Shelton, 2014).

Na avaliação das intervenções em Saúde Coletiva, os ECR geralmente não são suficientes como força motriz para a tomada de decisão. Usualmente, a triangulação de diferentes metodologias de pesquisa se condensam com as evidências científicas produzidas por estudos epidemiológicos de elevada validade interna, de modo a embasar as decisões sobre intervenções em saúde (Victora, Habitch & Bryce, 2004; Hatt et al., 2015).

A OMS recomenda a incorporação de um sistema padronizado de graduação, análise, desenvolvimento e avaliação das evidências (***G****rading of* ***R****ecommendatons* ***A****ssessment,* ***D****evelopment and* ***E****valuaton* - Sistema GRADE) por ocasião do estabelecimento de recomendações em saúde pública, bem como no processo de tomada de decisões em saúde (WORLD HEALTH ORGANIZATION, 2012). As orientações do Sistema GRADE, por exemplo, incluem e destacam a importância dos estudos observacionais e pesquisas qualitativas na produção do conhecimento e evidências (Baslshem et al., 2011).

Dentre as estratégias sugeridas para o estímulo ao uso de evidências na tomada de decisão, destacam-se as revisões sistemáticas (RS), que são estudos secundários que utilizam métodos estruturados e rigorosos para examinar, comparar e sintetizar as evidências empíricas relevantes a uma questão de pesquisa específica (Cook, Mulrow & Haynes, 1997; El Dib, Atallah & Andriolo, 2007; Brannon, Taylor & Coates, 2014). Tem por objetivo localizar, avaliar criticamente e interpretar todos os estudos disponíveis para uma questão de pesquisa, área do conhecimento ou fenômeno de interesse (Brasil, 2014).

Ao analisar toda a evidência disponível por meio de um processo transparente, explícito, reprodutível, rigoroso e abrangente, as RS reduzem os riscos de viés e erros randômicos (Cook, Mulrow & Haynes, 1997; Brannon, Taylor & Coates, 2014; Threlfall et al., 2015). As RS permitem a identificação da natureza e da qualidade das evidências disponibilizadas por estudos primários, a consistência do efeito estudado, e a evidência de causalidade, especialmente quando os resultados de múltiplos estudos seja controversa ou conflitante (Brannon, Taylor & Coates, 2014).

A despeito das RS contribuírem sobremaneira com a construção do conhecimento na SCBE, reduzindo o erro e melhorando a acurácia na tomada de decisões em saúde

(Ammerman, Smith & Calancie, 2014; Hatt et al., 2015; Threlfall et al., 2015), diversos autores sugerem que delineamentos alternativos e inovadores devam ser empregados e testados (Victora, Habitch & Bryce, 2004; Buendía-Rodríguez & Sánchez-Villamil, 2006; Ammerman, Smith & Calancie, 2014). Dentre estes, destacam-se os delineamentos randomizados e não-randomizados tipo cluster e as pesquisas qualitativas (Buendía-Rodríguez & Sánchez-Villamil, 2006), assim como as revisões relacionadas a fatores de risco e prognósticos, que incluem estudos observacionais comparativos, nos quais o pesquisador coleta dados, observando e comparando os eventos enquanto eles acontecem (Stroup et al., 2000; Brasil, 2014)

A Saúde Coletiva Informada pelas Evidências: avanços e obstáculos

A Saúde Coletiva propõe intervenções articuladas de promoção, proteção, recuperação e reabilitação da saúde com base em uma abordagem multidisciplinar (Souza, 2014), com foco nas populações e comunidades (ao invés de em indivíduos), mediante intervenções multicomponentes (ao invés de intervenções únicas), analisando não só os desfechos, mas igualmente o processo causal, teorias e crenças das comunidades (Buendía-Rodríguez & Sánchez-Villamil, 2006).

Neste contexto, é ímpar que sua complexidade encontre reflexo e respaldo nos meios particulares de apropriação e emprego das evidências científicas. De modo que as evidências científicas não determinam as ações e necessidades sociais de saúde, o emprego do conceito de “Saúde Coletiva Informada pelas Evidências” (SCIE) mostra-se mais apropriado do que o termo SCBE.

Ainda que as evidências constituam apenas um dos elementos no processo decisório, o uso sistemático das evidências oriundas dos resultados de pesquisas neste processo é escasso e incipiente, e representa um desafio para as políticas de saúde, principalmente em países em desenvolvimento (Dias et al., 2015).

Com o objetivo de promover mecanismos que facilitem o uso regular dos resultados de pesquisas na tomada de decisão, respeitando as necessidades locais e o uso racional dos recursos disponíveis, a OMS e a OPAS conceberam uma iniciativa inovadora, denominada *The Evidence informed policy network* (EVIPNet). A rede tem estado presente em 12 países latino-americanos desde o ano de 2012 (incluindo o Brasil), agindo como

facilitadora na revisão de políticas públicas, bem como na condução de diálogos deliberativos, como na prevenção e controle da Dengue no espaço urbano e no papel da atenção primária na abordagem compreensiva do manejo de doenças crônicas não-transmissíveis (Moat & Lavis, 2014).

Independente do contexto de desenvolvimento de uma sociedade, o contínuo desenvolvimento de inovações tecnológicas pode representar um problema para a gestão em saúde: à medida que a rápida difusão da informação técnico-científica (sob a influência da indústria, principalmente) cria fortes demandas pela incorporação destas inovações, os custos dos sistemas de saúde (públicos e/ou privados) tendem a aumentar significativamente. Infelizmente, uma inovação tecnológica não necessariamente representa economia de recursos ou incremento perceptível nos desfechos de interesse para a coletividade.

Neste sentido, já a partir da década de sessenta, a comunidade internacional passa a identificar a necessidade de constituir processos válidos para auxiliar a tomada de decisão por parte dos gestores em saúde, assim como dos profissionais de saúde, das chefias de serviços, das organizações de pacientes, do sistema judiciário e dos ministros de saúde. Surge, então, o conceito de avaliação de tecnologias em saúde (ATS), que trata de ”*um processo que avalia e regula o uso das tecnologias em saúde, oferecendo subsídios técnicos baseados na melhor evidência científica*” (Brasil, 2006)

A ATS emprega como ferramenta fundamental a avaliação crítica da validade das pesquisas clínicas realizadas com a tecnologia de interesse (medicamentos, procedimentos, equipamentos e sistemas organizacionais e de suporte), é realizada exatamente mediante a revisão sistemática da literatura, seguida da análise do custo-efetividade da potencial incorporação desta tecnologia (Brasil, 2006)

No âmbito do Sistema Único de Saúde (SUS), a Lei nº 12.401/2011, que dispõe sobre a assistência terapêutica e a incorporação de tecnologias em saúde, atribuiu à Comissão Nacional de Incorporação de Tecnologias (Conitec) a função de incorporação, exclusão ou alteração dessas tecnologias no SUS.

A criação da Rede Brasileira de Avaliação de Tecnologias em Saúde (Rebrats), institucionalizada pela Portaria nº 2.915, de 12 de dezembro de 2011, foi outro marco importante no estabelecimento de diretrizes públicas voltadas à geração e à síntese de evidências científicas no campo de ATS. A Rebrats é constituída por uma rede de centros

colaboradores e instituições de ensino e pesquisa, e encampou o trabalho de padronização e elaboração de diretrizes metodológicas para a ATS no país.

Realizar uma adequada avaliação crítica da validade, integridade e aplicabilidade das evidências pertinentes a intervenções em Saúde Coletiva pode ser desafiador: nem sempre os processos de randomização, sigilo da alocação e cegamento são factíveis nestes delineamentos, e os potenciais vieses de informação e contaminação dos grupos controle/intervenção, aliados ao elevado risco de abandono podem influenciar a avaliação da efetividade, usualmente comprometem a qualidade final destes estudos (Buendía-Rodríguez & Sánchez-Villamil, 2006). Ainda assim, a SCIE é fonte importante de informação para otimizar o uso dos recursos em saúde, e assegurar que a tomada de decisão esteja baseada em práticas efetivas e corroboradas.

CONSIDERAÇÕES FINAIS

A despeito dos conceitos "baseados em evidência" terem evoluído, desde seus primeiros registros, de um foco no cuidado individual com a saúde, para uma conceituação mais ampla de PBE, sua transposição para o campo de práticas da Saúde Coletiva não tem sido direta. Em Saúde Coletiva, os meios de apropriação e emprego das evidências mostram-se muito mais complexo do que o envolvimento nas decisões clínicas, pois envolvem pressupostos de plausabilidade e adequação nem sempre observados em intervenções individuais, bem como avaliações de impactos orçamentários e políticos.

Ainda assim, o campo beneficia-se da aproximação observada na SCIE, em particular, na tomada de decisões em saúde. Iniciativas globais e regionais tem demonstrado a importância desse olhar, com vistas a otimizar práticas e tornar as ações em saúde mais efetivas. Os autores verificaram, do mesmo modo, a escassez de estudos sobre o uso das evidências científicas em Saúde Coletiva, indicando a necessidade de mais estudos na área.

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2.2 Capítulo 2: Prevenção de infecções em idosos

A senescência, ou envelhecimento fisiológico humano, trata de um conjunto de alterações estruturais e funcionais do organismo que se desenvolvem progressiva e especificamente em função do avançar da idade, acumulando danos em níveis moleculares, celulares e orgânicos, que resultam em redução ou desregulação funcional e aumento da chance de adoecimento e óbito.[22,23]

Observa-se, por exemplo, a perda e ruptura de barreiras epiteliais protetoras na pele, redução do *clearence* mucociliar no trato respiratório, aumento da capacidade de invasão dos sistemas pulmonares, gastrointestinais e urológicos por organismos patogênicos, redução na capacidade de mastigação e distúrbios de deglutição, dentre outras disfunções.[22,24]

Dentre várias destas complexas alterações, muitas com ações deletérias, outras com intenções adaptativas, as modificações do envelhecimento no sistema imune destacam-se pela importância na gênese da morbimortalidade deste segmento etário.[8,24] A imunosenescência afeta não apenas a imunidade adaptativa, mas também a imunidade inata.[9,23–25] A desregulação ou comprometimento da imunidade tem sido assumida como causa plausível para o aumento da susceptibilidade e sensibilidade da população idosa as doenças infecciosas, bem como a baixa resposta à vacinação.[7,8,13,24]

A imunosenescência está associada ao declínio no fenótipo e função das células T e das células "*natural killer*" (NK).[7,8] A involução tímica no idoso contribui para a redução no número de células T *naive* e redução no número de linfócitos, cujo espaço é preenchido pela expansão e acúmulo de células T de memória senescentes e não-funcionais.[7,8,24]

O declínio na função das células T-CD8 (possível relação com o encurtamento dos telômeros) desencadeia falhas no processo de sinalização e ativação das células T.[7] Mudanças fenotípicas também são observadas nas células NK, que mesmo em maior número, apresentam reduzida capacidade de expressão de receptores de ativação, bem como reduzida citotoxicidade.[7,8,24]

Os fatores intrínsecos (involução tímica, deterioração do estroma da medula óssea e da imunidade inata e adaptativa), bem como fatores extrínsecos (especialmente ligados a infecção crônica pelo citomegalovírus e deprivação estrogênica da menopausa, por exemplo) podem influenciar profundamente as alterações do sistema imune associadas a idade.[7] Alguns dos mecanismos envolvidos na relação da imunosenescência com os perfis de morbidade e mortalidade por causas infecciosas ainda permanecem pouco elucidados.[7,9,26] Não está clara, por exemplo, se a susceptibilidade dos idosos a infecções é um fenônmeno dependente de contextos de situação, se há real contribuição da idade e do sexo, o papel dos contatos familiares, etc.

Apesar da evolução tecnológica, sanitária e assistencial que acompanhou e permitiu o envelhecimento populacional, as infecções em pacientes idosos permanecem como um sério problema de saúde pública.[27–29] Em países em desenvolvimento, a pobreza, a deficiência de infra-estrutura e a falta de recursos para suprir as demandas clínicas do segmento idoso amplificam a possibilidade de transmissão de patógenos e a susceptibilidade de contaminação entre idosos.[28]

Nos Estados Unidos estima-se aproximadamente 21 milhões de hospitalizações secundárias a infecções na população de idosos entre os anos de 1990 e 2002, com uma tendência de aumento nas taxas de hospitalização entre os muito idosos (85 anos ou mais) com o passar dos anos.[4] Apenas no ano de 2012, neste mesmo país, estimou-se mais de 3,1 milhão de visitas de idosos a unidades de emergência por causas infecciosas, com subseqüente hospitalização e óbito de aproximadamente 120.000 pacientes em unidades geriátricas.[3] O impacto destas visitas a unidades de emergência foi superior ao observado para admissões por insuficiência cardíaca congestiva e infarto agudo do miocárdio combinadas no mesmo período.[3]

As infecções em idosos são mais freqüentes, mais graves e potencialmente associadas a maiores taxas de mortalidade e declínio funcional do que em pacientes jovens.[6] As taxas de hospitalização e óbito por infecções bacterianas entéricas secundárias a contaminação de alimentos é visivelmente superior entre as pessoas com mais de 65 anos nos Estados Unidos,[30] fato que muito provavelmente se replica em outras nações desenvolvidas e em desenvolvimento.

De fato, acredita-se que os dados de mortalidade geral secundários a causas infecciosas sejam subestimados, tendo em vista que são, na maioria das vezes,

levantamentos realizados com base na notificação mediante a comunicação de atestados de óbitos; estudo comparando estas notificações com o resultado de autópsias sugere que as causas infecciosas possam, em países como os Estados Unidos, representar na verdade 20 a 30% das causas de óbito em idosos.[31]

As pneumonias e infecções do trato respiratório inferior destacam-se como algumas das principais causas de morbidade e mortalidade entre os idosos.[32–34] Em estudo de análise populacional no Reino Unido, identificou-se sete vezes mais casos de pneumonia adquirida na comunidade (PAC) no subgrupo de idosos com 85 a 89 anos quando comparados com os indivíduos entre 65 e 69 anos, bem como incidência 70% superior no quintil com maior privação econômica, social e funcional.[32] Um estudo de coorte alemão com quase 500 pacientes idosos com diagnóstico de PAC encontrou taxas de mortalidade superiores a 21% em um ano.[33]

Nos idosos assistidos em unidades de longa permanência, a transmissão e as taxas de morbimortalidade por infecções podem ser ainda maiores. Estudos descrevem taxas de prevalência pontual de 4,3%, com comprometimento mais freqüente dos tratos urinário (28%), respiratório (25,5%), pele (15,5%) e gastrointestinal (6,8%).[35] Outros autores descrevem densidade de incidência de infecções de 3,4 por 1000 pacientes dia em instituições para idosos no Brasil,[36] atribuindo as multimorbidades, ao declínio funcional e cognitivo, ao uso de dispositivos invasivos (como cateteres e sondas), ao convívio em ambientes restritivos e pouco ventilados e a imunosenescência o aumento da susceptibilidade neste grupo.[37]

2.3 Capítulo 3: Probióticos na prevenção de infecções

Os probióticos são microrganismos vivos que, quando administrados em quantidade suficiente, exercem efeitos benéficos à saúde do hospedeiro.[38] Apesar de utilizados desde a antiguidade, os conceitos mais modernos dos probióticos foram introduzidos na ciência com os trabalhos de *Elie Metchnikoff* (1845-1916), com base nas observações de um microbiologista búlgaro (Professor *Stamen Grigorov*) sobre os efeitos benéficos à saúde de um iogurte; posteriormente seu componente ativo foi identificado como sendo o *Lactobacillus dellbrueckii ssp. Bulgaricus.*[39]

Os microrganismos probióticos são usualmente membros dos gêneros *Lactobacillus* (L.), *Bifidobacterium* (B.), Streptococcus (S.), e a levedura *Saccharomyces boulardii*, apesar de outros gêneros serem descritos com alguma freqüência.[23] O que os diferencia de microrganismos não probióticos e bactérias patogênicas é exatamente a identificação (experimental e/ou clínica) de que determinada cepa produza efeitos benéficos ao hospedeiro se ingerida em quantidade suficiente.

O processo de senescência produz diversas modificações no trato gastrointestinal do idoso que aumentam a susceptibilidade a infecções: (a) redução no número de microrganismos da flora normal do cólon (incluindo bifidobactérias), o que predispõe ao aumento de outras bactérias proteolíticas; (b) redução na secreção de IgA de mucosa no lúmen intestinal em resposta a presença de bactérias, vírus e fungos; (c) redução na resposta das células T dos tecidos linforreticulares associados ao intestino, dentre outras.[23]

Aos probióticos têm sido creditadas ações imunomoduladoras que incluem melhora na capacidade de barreira do epitélio do lúmen intestinal, aumento na capacidade de adesão da mucosa intestinal (e concomitante inibição da adesão de patógenos), exclusão competitiva de microrganismos patogênicos, produção de substâncias anti-microrganismos e ação moduladora no sistema imune.[40] Além destas propriedades, estudos têm evidenciado que a administração de probióticos poderia proteger cobaias jovens de infecções por *Salmonella typhimurium* [41] e *Listeria monocytogenes*[42], reduzindo a chance de translocação de patógenos para o baço e fígado, enquanto aumenta a atividade fagocítica, proliferação linfocítica e resposta antigênica de mucosa.

Diferentemente de outros componentes alimentares que ofereçam vantagens funcionais ou exibam, em estudos clínicos, benefícios à saúde humana, os probióticos exibem uma característica única: eles podem ser patenteados. Enquanto vitaminas ou minerais raramente dispõe desta vantagem comercial, novas cepas bacterianas podem ser desenvolvidas de modo único e suficientemente viável para tornar um produto altamente lucrativo.[43]

Por exemplo, o preço de varejo do litro de um dos probióticos mais conhecidos no mercado mundial (Yakult®) é de 4,79 euros, comparados a 0,75 euros de um iogurte regular;[43] o mercado global de probióticos ultrapassou, em 2013, a movimentação de U$ 19,6 bilhões.[44]

Algumas controvérsias envolveram, anteriormente, pesquisas e tentativas de regulamentação do uso de probióticos e seu emprego como produtos com alegações de benefícios para a saúde.[43,45–48] De acordo com a legislação da União Européia (UE), por exemplo, é ilegal afirmar ao consumidor que um alimento pode prevenir, tratar ou curar uma doença.[43] Pode não fazer sentido, tendo em vista que a ciência tem fortes evidências do uso de componentes alimentares para prevenir defeitos do tubo neural ou curar escorbuto (apenas para citar dois exemplos), mas perante a legislação da UE, as exigências para a aprovação dos "*health claims*" para alimentos como os probióticos podem ter sido utilizadas como uma das justificativas para a falta de estudos com melhor validade externa.

Outra alegação envolve o tipo de desfecho analisado nos ensaios clínicos com probióticos, que freqüentemente dedicam-se mais a analisar evidências sobre potenciais papéis imunomodulares (sobre a ação em células imunes) do que ao estudo da efetiva redução de desfechos significantes, como a redução na incidência de infecções, redução na duração de diarreia, melhora da constipação, controle ou redução de episódios rinite alérgica e asma, etc.[43]

De fato, metanálises anteriores observaram boa efetividade dos probióticos na redução de enterites agudas em crianças pré-escolares, bem como redução da freqüência de enterocolite necrotizante em recém nascidos pré-termo.[48,49] Outra revisão sistemática encontrou evidências de proteção e redução no número episódios de infecções agudas de vias aéreas superiores em crianças, bem como redução no uso de antibióticos, quando comparado ao placebo.[50]

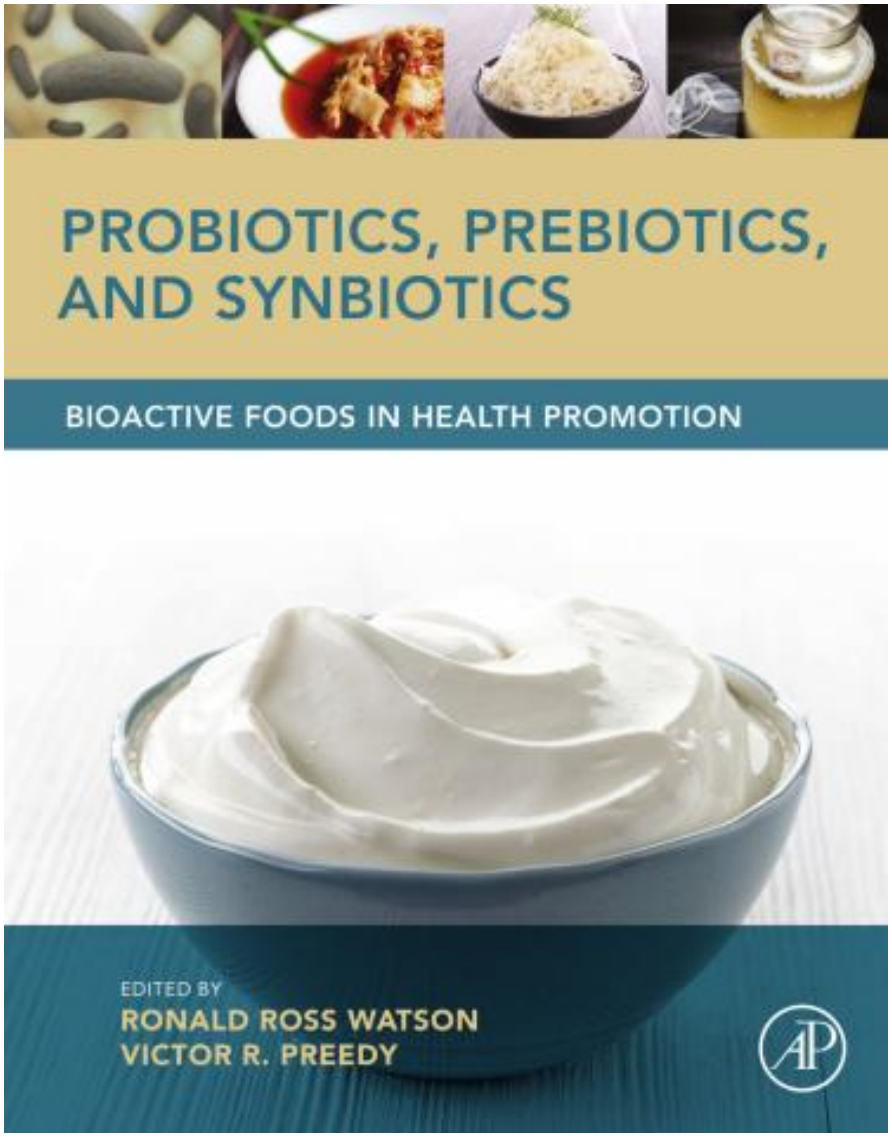
ECR com idosos que receberam suplementação com probióticos por três meses (durante o período de inverno) identificou redução na duração média por episódio de infecção quando comparado ao placebo (6.5 *versus* 8 dias no grupo controle) e redução na freqüência dos episódios de doenças infecciosas comuns, principalmente para infecções de vias aéreas superiores.[17]

De modo similar, um ECR com idosos que receberam iogurte suplementado com *L. bulgaricus* e *S. thermophilus* por doze semanas observou redução significativa na freqüência de episódios de resfriado comum e infecções por vírus influenza, quando comparados ao placebo.[51]

Outros estudos, entretanto, não conseguirem reproduzir estes achados. Allen e colaboradores,[19] investigando o desenvolvimento de infecções por *Clostridium difficile* em uma grande amostra de idosos, não encontraram evidência de proteção. Do mesmo modo, outro ECR conduzido com 737 idosos saudáveis não encontrou diferença significativa na frequência, duração ou gravidade dos sintomas de infecções respiratórias quando comparado ao placebo.[20]

Apesar de existirem ensaios clínicos randomizados envolvendo probióticos e idosos, e de serem conhecidos os impactos da imunosenescência e do envelhecimento fisiológico na predisposição e aumento da susceptibilidade deste segmento etário a infecções, não existe, até o momento, nenhuma RS publicada que permita identificar se o uso de probióticos neste segmento etário ofereça (ou não) benefícios na prevenção de infecções.

2.4 Capítulo 4: Publicação 1 – "*Evidence and rationale for probiotics to prevent infections in the elderly*"



Probiotics, Prebiotics, and Synbiotics

Bioactive Foods in Health Promotion

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Chapter 50

Evidence and Rationale for Probiotics to Prevent Infections in the Elderly

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1 BACKGROUND

Probiotics are viable or live microorganisms that confer a health benefit to the host when administered or ingested in sufficient amounts, with or without synergistic combinations (as probiotics and prebiotics, also defined as symbiotics) (Bermudez-Brito et al., 2012; Patel et al., 2014; Reid et al., 2003). Different from some strains of lactic acid bacteria, probiotics can survive transit through the gastrointestinal tract, which allows them to interact with gut commensal microbiota and with intestinal lymphoid and epithelial cells (Nova et al., 2007).

Typical doses used in studies ranged from 10^7 to 10^{10} colony-forming units/g (Bermudez-Brito et al., 2012; World Health Organization, 2001). Probiotics can be found in commercially available preparations or manipulated products and have shown clinical potential against some immune and infectious illnesses (Duncan and Flint, 2013; Nova et al., 2007; Patel et al., 2014; Reid et al., 2003).

Several probiotics are available worldwide; the most commonly reported strains are: the *Lactobacillus* species *acidophilus, bifidum, brevis, buchneri, bulgaricus, casei* subsp. *paracasei,* and subsp. *tolerans, caucasicus, coryniformis, crispatus, delbrueckii, fermentum, gasseri*, (GG), *helveticus, johnsonii, lactis, leichmannii, paracasei, plantarum, reuteri, rhamnosus*, and *salivarius*; the *Bifidobacterium* species *animalis, bifidum, breve, clausii, infantis, lactis*, and *longum*; the *Saccharomyces* species *boulardii, cerevisiae*, and *cerevisiae boulardii*; and the *Enterococcus* species *faecalis* and *faecium*. There are also the *Streptococcus* species *mitis, oralis, rattus, salivarius, sanguis, thermophilus*, and *faecium* and the *Bacillus* species *clausii, coagulans, licheniformis, oligonitrophilus, stearothermophilus*, and *subtilis*.

The probiotics' mechanisms of action include improvement of the epithelial barrier, competitive adhesion to intestinal mucosa (thereby inhibiting pathogen adhesion), modulation of the immune system, and production of antipathogen substances (Bermudez-Brito et al., 2012; Kotzampassi and Giamarellos-Bourboulis, 2012; World Health Organization, 2001). These biological activities seem to benefit health at different stages in life (Duncan and Flint, 2013; Kotzampassi and Giamarellos-Bourboulis, 2012; Nova et al., 2007).

In all age groups, the metabolites produced by gut microbiota, their interaction with food and pathogens appear to influence the health and disease balance; however, in the elderly, their impact may be more important (Duncan and Flint, 2013; Nova et al., 2007; Patel et al., 2014). With aging, the diversity of gut microbiota appears reduced, with an increase in *Enterobacteriaceae* and *Proteobacteria*, and a reduction in the number of *Bifidobacterium* species (Duncan and Flint, 2013). These modifications may be even greater in elderly residents of long-term care facilities; they usually have a higher proportion of *Bacteriodetes* and decreased *Firmicutes* numbers, compared to community-dwelling elderly populations (Biagi et al., 2012; Britton and McLaughlin, 2013; Claesson et al., 2012).

The strict anaerobic bacteria in the gut ferment the ingested carbohydrates that escape the digestion process of the host, to form short-chain fatty acids, whose major metabolites are acetate, butyrate, and propionate (Duncan and Flint, 2013). Propionate has a gluconeogenic function, and acetate is mainly metabolized by peripheral tissues, whereas butyrate is one of the principal energy sources for colonic epithelial cells (Britton and McLaughlin, 2013; Duncan and Flint, 2013). Although changes in the aging gut microbiota require further investigation, it appears that butyrate levels are lower in the aged than in healthy younger adults and that lactate accumulation might be indicative of microbial imbalance (Duncan and Flint, 2013; Lakshminarayanan et al., 2013).

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The modifications of the aging gut microbiota are not the only explanation for why older persons are more susceptible to infections. They have a compromised ability to ward off infectious agents due to changes in nonadaptative immunity, comorbid chronic diseases, effects of multiple chronic medications, malnutrition, and functional deficits (Duncan and Flint, 2013; Lakshminarayanan et al., 2013; Nova et al., 2007; Richards, 2002). The immune system's functional dysregulation has been assumed to be the primary plausible cause for the elderly's increased susceptibility to infectious diseases (Liang and Mackowiak, 2007; Nova et al., 2007; Steens et al., 2014).

Immunosenescence seems to affect both innate and adapttive immunity (Dewan et al., 2012; Ongrádi and Kövesdi, 2010; Pawelec et al., 2010). Immunosenescence, when combined with a breakdown of epithelial barriers, appears to facilitate invasion by pathogenic microorganisms (Ongrádi and Kövesdi, 2010; Pae et al., 2012).

Compared to young hosts, the efficiency of migration of antigen-presenting cells and the stability of contact with T cells (immunological synapse) are reduced in the elderly. This results in poor activation and differentiation of T cells. Thymic output also decreases in the aged host, which further compromises the immune response; B cell output from the bone marrow is also adversely affected by aging. T-B interactions occur less efficiently, fewer germinal centers are formed, and poor differentiation leads to poor antibody responses (Salam et al., 2013).

With aging, it appears that the absolute number of B cells and the ratio of CD4+/CD8+T lymphocytes declines with a relative increase in natural killer cells (McElhaney and Effros, 2009). The quality of the humoral immune response is less optimal in the elderly (Pae et al., 2012), and cellular functions and regulatory mechanisms are also impaired, including proliferation and differentiation in response to antigen activation (Boraschi et al., 2010).

Probiotics are believed to modulate immune functions through interaction with lymphoid and epithelial gut cells, affecting mucosal surfaces via mucosal immune systems (Bermudez-Brito et al., 2012; Pae et al., 2012). One of the benefits related to consuming probiotics, especially bifidobacteria, is the production of metabolites of fermentation, including acetate and vitamins (such as folate), that can inhibit pathogens (Bermudez-Brito et al., 2012; Duncan and Flint, 2013). The health benefits of probiotics, however, seem to be strain-specific, and the properties attributed to one strain may not be automatically extrapolated to all other species (Duncan and Flint, 2013).

Previous investigations suggested probiotics for prevention against infections, especially in children and older persons (Pae et al., 2012). There is evidence that consuming probiotics causes an increase in the number of probiotic bacteria in the intestines and feces. However, cells' markers are less credible health outcomes than disease occurrence. Notably, previous studies used surrogates instead of clinically significant outcomes, which may have produced spurious results (Katan, 2012).

We will now present the available evidence that supports the use of probiotics as a potential intervention to prevent infections, focusing on their use in older age groups and to prevent rather than treat infections.

Noncommunicable causes of death (such as cancer, heart failure, and diabetes) are predominant in the elderly in developed countries; however, in the developing world, infectious diseases impact morbidity and mortality more. In Europe and in the United States, almost 5% of the elderly population will die as a consequence of infectious diseases, whereas this proportion in Africa is expected to be approximately 20% (Videcnik Zorman et al., 2013; World Health Organization, 2001). Consequently, infections in the elderly will contribute importantly to societal and health costs in developing countries in the coming decades (Gavazzi et al., 2004).

2 GASTROINTESTINAL INFECTIONS

In this section, we will evaluate the available evidence for the effects of probiotics on the most common gastrointestinal infections, and those gastrointestinal infections in which effectiveness and efficacy have been studied appropriately.

Antibiotic-associated diarrhea (AAD) may be defined as otherwise unexplained diarrhea with an onset either after starting antibiotic therapy or less than 6-8 weeks after finishing treatment for community and/or hospital-acquired infections (de Souza and Jorge, 2012). These episodes can affect approximately 5-39% of patients using antimicrobial agents (Friedman, 2012; Pozzoni et al., 2012; de Souza and Jorge, 2012; Tabasco et al., 2012). The incidence varies according to the type of antibiotic used, the duration of treatment, host health and age, and exposure to pathogens (Hickson, 2011; Tabasco et al., 2012).

The nonpathogenic gut microbiota (especially anaerobic bacteria) plays a pivotal role in controlling the microbial population in the human gastrointestinal tract. Antibiotics can cause an imbalance of this microbiota, disrupting the normal colonic microflora and reducing the fermentation of indigested carbohydrates (Hickson, 2011).

Consequently, patients can develop either osmotic diarrhea or diarrhea caused by pathogenic bacteria (D'Souza et al., 2002; Hickson, 2011; Hickson et al., 2007). In the first mechanism, decreased metabolism of fermentable carbohydrates increases nonabsorbable carbohydrates, which elevates the luminal osmotic pressure, and thereby reduces water absorption

from the gut. Second, a breakdown in the colonization resistance creates a favorable environment for the growth of pathogens that cause AAD (D'Souza et al., 2002; Hickson et al., 2007; Pozzoni et al., 2012; de Souza and Jorge, 2012).

Due to how aging changes the gut microbiota, elderly persons are at greater risk of developing AAD (Hickson, 2011). The mechanism of action of probiotics in AAD is related to enhancement of the mucosal barrier by secreting mucins, increase of tight junctions, colonization resistance, production of bacteriocins, and increased production of secretory IgA and IL-10 (Friedman, 2012).

Hickson et al.(2007) studied the efficacy of a *Lactobacillus* probiotic drink on the prevention of AAD in a sample of older persons and found that probiotic consumption reduced the incidence of AAD, with an absolute risk reduction of 21.6% (6.6-36.6%); to prevent one episode of AAD, the number needed to treat was five. Gao et al. (2010) randomized inpatients aged 50-70 years to one of three groups: two probiotic capsules (*Lactobacillus acidophilus CL1285+Lactobacillus casei LBC80R*) per day; one probiotic capsule and one placebo capsule per day; or two placebo capsules per day. They found that probiotics reduced the risk of AAD and, in particular, *Clostridium difficile* diarrhea (CDD) in hospitalized patients on antibiotics.

Beausoleil et al.(2007) randomized 89 inpatients (mean age 70 years) to receive either lactobacilli-fermented milk or a placebo daily. The authors concluded that the daily administration of lactobacilli-fermented milk was safe and effective to prevent AAD in hospitalized patients (OR 0.34, 95% CI = 0.125, 0.944; $p = 0.05$). Although several trials used *Lactobacillus* strains, the largest body of evidence is for *S. boulardii* (Hickson, 2011).

A meta-analysis of randomized controlled trials (RCTs) conducted by D'Souza et al. (2002) suggested that probiotics could be used to prevent AAD and that *S. boulardii* and lactobacilli had potential to be used to prevent this clinical condition. A meta-analysis of RCTs (McFarland, 2006) examined data from trials using mixtures of probiotics (seven trials) and *L. rhamnosus* (six trials) and found that the relative risks of developing an AAD were 0.51 (95% CI = 0.38, 0.68; $p < 0.0001$) and 0.31 (95% CI = 0.13, 0.72; $p = 0.006$).

Furthermore, a systematic review (McFarland, 2010) of 10 RCTs evaluated the efficacy of *S. boulardii* to prevent AAD and found a relative risk of 0.47 (95% CI = 0.35, 0.63; $p < 0.0001$) for this intervention. These results, however, are related to trials in which elderly were not the primary age group.

An economic evaluation of a probiotic (*Lactobacillus paracasei* ssp. *paracasei*) to prevent ADD showed that this intervention generated an estimated mean savings of £339 per hospitalized patient over the age of 65 years treated with antibiotics compared to no preventive probiotic.(Lenoir-Wijnkoop et al., 2014)

A multicenter pragmatic efficacy trial (Allen et al., 2013) developed to test a multistrain preparation of lactobacilli and bifidobacteria to prevent AAD and CDD in older inpatients (the PLACIDE study) found no evidence of efficacy in prevention of AAD or CDD.

A meta-analysis of RCTs of probiotic efficacy for overall gastrointestinal diseases found that probiotics (11 species were analyzed) were generally beneficial for prevention and treatment, with a RR = 0.58 (95% CI = 0.51, 0.65) (Ritchie and Romanuk, 2012). However, efficacy was not observed for traveler's diarrhea or necrotizing enterocolitis, Significant efficacy was observed for all of the age groups identified in the review, and the type of disease and probiotic strain was the most important factors related to the outcomes (Ritchie and Romanuk, 2012).

Finally, a Cochrane systematic review and meta-analysis of 23 RCTs, including 4213 patients, found moderate quality evidence, suggesting that probiotics are both safe and effective for preventing CDD, reducing the risk by 64% (Goldenberg et al., 2013).

3 COMMON COLD AND AIRWAYS INFECTIONS

Older persons experience more severe and frequent community-acquired respiratory infections. Immunosenescence is probably the main factor involved in this increased susceptibility, because it decreases protection against new pathogens in this age group (Guillemard et al., 2010). Several infections, including the common cold, acute otitis media, acute sinusitis, acute pharyngitis, and laryngotracheobronchitis, are commonly described as acute upper respiratory tract infections (URTIs) (Hao et al., 2011). Community- and nosocomial-acquired pneumonia are the primary acute lower respiratory tract infections (LRTIs), and together with influenza, they represent the fourth most common cause of death among the aged (Guillemard et al., 2010).

As stated by Guillemard et al. (2010), optimal success in preventing and controlling infectious diseases in the elderly requires not only more effective antimicrobial agents and vaccines, but also strategies to increase the immune response effectiveness. Functional foods, as probiotics, have been studied, along with improvement in living conditions, promotion of physical activities, and better nutrition (Guillemard et al., 2010).

The effects of probiotic bacteria on the prevention of URTI and LRTI have been studied in RCTs (de Vrese et al., 2006; Fujita et al., 2013; Guillemard et al., 2010; Kekkonen et al., 2007; Makino et al., 2010; Turchet et al., 2003; Van Puyenbroeck et al., 2012).

In a nonrandomized study, Makino et al.(2010) evaluated the effect of *Lactobacillus delbrueckii* spp. *bulgaricus* OLL1073R on resistance to the common cold. The authors concluded that the consumption of fermented yogurt containing probiotics reduced the risk of acquiring the common cold in elderly Japanese individuals. In six other RCTs reviewed by Nahas and Balla (2011) involving 1.766 healthy adults and elderly,(de Vrese et al., 2005; Kekkonen et al., 2007; Tiollier et al., 2007; Tubelius et al., 2005; Turchet et al., 2003; Winkler et al., 2005) the number of common colds was significantly reduced in only one trial (Winkler et al., 2005). Other outcomes were evaluated, such as symptom severity and duration of common cold episodes (reduced in one trial) (de Vrese et al., 2005).

Guillemard et al.(2010) conducted a multicenter RCT to examine the effects of a dairy product containing *Lactobacillus casei* DN-114001on the prevention of common infectious diseases (URTIs and gastrointestinal tract infections) in the elderly. They concluded that the intervention was associated with a decreased duration of the infectious episodes, compared to the control group, especially for URTIs.

Van Puyenbroeck et al.(2012) studied the protective effect of an intervention with *L. casei Shirota* in elderly long-term care residents and found no statistically or clinically significant effect on URTIs (OR of probiotic: 0.8715-95% CI=0.6168, 12887).Fujita et al.(2013), who performed a multicenter trial to investigate the preventive effects of the same strain used by Van Puyenbroeck et al.(2012) in an elderly population, also found no effect (incidence of URTI episodes - Intervention: 0.0066; placebo: 0.0048; $p=0.89$).

Hao et al. (2011) conducted a Cochrane systematic review of RCTs to assess the effectiveness and safety of probiotics to prevent acute URTIs. Fourteen RCTs were included, and the authors found that probiotics were better than the placebo in reducing the number of patients experiencing episodes of URTIs (OR 0.58-95% CI=0.36, 0.92). The authors claimed, however, that the results cannot be extrapolated to elderly persons due to the absence of appropriate data. They identified three RCTs (Guillemard et al., 2010; Makino et al., 2010; Turchet et al., 2003) comparing probiotics to placebo in older adults, however, two studies that did not fulfill inclusion criteria were excluded (Guillemard et al., 2010; Turchet et al., 2003).

Few studies have evaluated probiotics for prophylaxis of LRTIs. Morrow et al. (2010) conducted a trial to determine if *L. rhamnosus* could reduce the incidence of ventilator-associated pneumonia (VAP). They found that patients treated with this intervention were significantly less likely to develop VAP, than the placebo group (40.0% vs. 19.1%; $p=0.007$).

Future RCTs should focus on older persons and consider better study designs that incorporate adequate blinding and allocation concealment, and assess adequate primary outcomes (e.g., number of infectious episodes and the mean duration, instead of laboratory surrogates) (Hao et al., 2011).

4 GENITOURINARY INFECTIONS

Although asymptomatic bacteriuria in the elderly should not be treated with antibiotics, urinary tract infections (UTIs) and recurrent UTIs are major problems and common bacterial infections in young and older women (Raz, 2011; Uehara et al., 2006). Estrogen deficiency seems to play an important role in the development of bacteriuria and UTI, and the efficacy of estrogen to prevent UTI is supported by good evidence (Raz, 2011).

Some risk factors for recurrent UTI in postmenopausal women are incontinence, presence of cystocele, postvoiding residual urine, and a history of UTI before menopause (Raz et al., 2000).

Strategies that are presently advocated or under investigation to prevent UTI include prophylactic antibiotics, functional foods (e.g., cranberry juice), vaccines, probiotics, and miscellaneous products (spermicides, hygiene products, etc.) (Uehara et al., 2006). Despite the risk of increased drug-resistant microorganisms, long-term antibiotic prophylaxis remains the most common method used to prevent UTI. Because the previous studies found a decreased survival associated with UTI, (Dontas et al., 1981; Nordenstam et al., 1986) it is imperative to study alternative strategies to prevent these infections.

Reid et al. (1985) studied *in vitro* the properties and efficacy of probiotics (lactobacilli) to prevent UTI in mice and showed that *L. casei* prevented the onset of UTI in 84% of the animals tested. In a prospective, clinical pilot study conducted by Uehara et al.(2006) to evaluate the safety and effectiveness of Lactobacillus vaginal suppositories to prevent recurrent UTI, the authors found a significant reduction in the mean number of recurrences (5 ± 1.6 episodes/year vs. 1.3 ± 1.2; $p=0.0007$).

In a case control study, Kontiokari et al. (2003) found that fermented milk containing probiotic bacteria appeared to be associated with a lower risk of UTI, among other dietary factors studied. Barraud et al. (2010) conducted a RCT to evaluate the preventive effects of probiotics in critically ill patients and found no difference between placebo and intervention groups in the risk for nosocomial UTI.

Beerepoot et al. (2012) compared probiotics and prophylactic antibiotics in a noninferiority preventive RCT in postmenopausal women with recurrent UTI and found that *L. rhamnosus* and *L. reuteri* did not meet the noninferiority criteria for UTIs when compared to trimethoprim-sulfamethoxazole.

Grin et al. (2013) conducted a systematic review of RCTs to determine the efficacy of Lactobacillus species to prevent recurrent UTI and found that the probiotic strains used were effective in preventing UTI in adult women. However, more RCTs are need because the patient population available for review was small.

Schwenger et al. (2010) are conducting a Cochrane systematic review of RCT on probiotics to prevent UTIs in adults and children. Their results will probably be available soon, reinforcing the available evidence for the effectiveness of probiotics to prevent UTI and recurrent UTI.

Homayouni et al. (2014) provided a review of the clinical evidence for the efficacy of probiotics to prevent and treat bacterial vaginosis (BV). The authors found that, although the results of different studies are controversial, most studies have been supported probiotics to prevent or treat BV, and no adverse effects have been reported.

5 FINAL CONSIDERATIONS

Although the gut microbiota is considered to play a pivotal functional role in human health (through nutritional, physiological, and immunological processes), the current knowledge, provided from several RCTs and systematic reviews, is very heterogeneous.

The ability of systematic reviews to provide definite answers about probiotics and infection prevention in the elderly are hampered by the following features of many available RCTs: broad exclusion criteria, diverse (and sometimes not useful) outcomes measures (surrogates instead of clinical meaningful outcomes, for instance), and the comparison of the efficacy of probiotics with placebo when other treatments are available.

As stated by Katan (2012), "a definitive, high quality study of the effect of probiotics on common infections will cost only a fraction of the funds currently spent on advertising." The gaps in the evidence for probiotics will only be filled when trials enroll patients from all age groups, irrespective of clinical status (healthy vs. nonhealthy), use specific interventions and evaluate clinically meaningful outcomes.

Based on the available evidence, it appears that probiotic ingestion is efficacious for the prevention of AAD and CDD (although recent RCTs found no differences) (Allen et al., 2013) and may be efficacious for gastrointestinal infections and URTIs, especially in children, (Hao et al., 2011) because the available evidence from clinical trials in the elderly is sparse.

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OBJETIVOS

3. Objetivos

- avaliar a efetividade e segurança do consumo de probióticos na prevenção de infecções em idosos.

MÉTODOS

4. Métodos

4.1 Descrição dos procedimentos

4.1.1 Delineamento do estudo e considerações éticas

Este trabalho adota como delineamento de estudo os métodos de uma Revisão Sistemática. Uma RS é um estudo secundário que utiliza métodos estruturados, sistematizados e rigorosos para examinar, comparar e sintetizar as evidências empíricas relevantes a uma questão de pesquisa específica.[52–54]

Ao analisar toda a evidência disponível por meio de um processo transparente, explícito, reprodutível, rigoroso e abrangente, as RS reduzem os riscos de viés e erros randômicos.[52,53,55] As RS permitem a identificação da natureza e da qualidade das evidências disponibilizadas por estudos primários, a consistência do efeito estudado, e a evidência de causalidade, especialmente quando os resultados de múltiplos estudos sejam conflitantes.[52]

A metanálise (MA) é a análise estatística dos resultados de diferentes estudos individuais, frequentemente mas não obrigatoriamente realizada em conjunto com uma RS, com o objetivo de integrá-los, combinando e resumindo seus resultados; com isso, uma MA é capaz de reduzir o desvio padrão e o intervalo de confiança, tornando o resultado mais confiável.[52–54]

Por tratar-se de um estudo que utiliza dados secundários, este trabalho foi submetido ao Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu – Universidade Estadual Paulista (Unesp), tendo sido dispensado pelo mesmo da obtenção de parecer ético (Anexo 4). O protocolo desta RS foi registrado e publicado na base de dados PROSPERO, sob o número CRD42014013707 (Anexo 3).

4.1.2 Pergunta de pesquisa e estratégia PICO

Para essa revisão sistemática estabeleceu-se a seguinte questão de pesquisa: “*O consumo de probióticos é efetivo e seguro na prevenção de infecções em idosos?*

Estruturou-se, do mesmo modo, os componentes do acrônimo PICO para essa mesma questão:

P	(pacientes)	Idosos (pessoas com 65 anos ou mais)
I	(intervenção)	Consumo de probióticos
C	(comparador)	Placebo
O	(desfecho)	Prevenção de infecções

4.1.3 Estratégia de busca

Durante a elaboração do protocolo e aplicação das estratégias de busca desta RS adotou-se as recomendações da Colaboração Cochrane, particularmente os princípios do "*Methodological Expectations of Cochrane Intervention Reviews"*.[56] Do mesmo modo, os métodos e resultados desta RS são apresentados obedecendo às recomendações do "*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*" (PRISMA).[57]

A estratégia de busca desta RS foi elaborada em parceria com a coordenadora de pesquisas, Sra Vitoria Lutje, do *Cochrane Infectious Disease Review Group (CIDRG)*. Em sua elaboração, considerou-se estratégias que permitissem a identificação de todos os estudos relevantes para a pergunta de pesquisa, independente de linguagem, período de publicação, ou *status* de publicação (publicado, não publicado, em andamento). As buscas eletrônicas foram conduzidas pelo pesquisador principal (PAW).

A estratégia foi composta pelas seguintes palavras-chave do *Medical Subject Headings* (MeSH), e seus principais sinônimos: *aged; aged, 80 and over; probiotics; lactobacillus; lactococcus; bifidobacterium; enterococcus; streptococcus; saccharomyces; e infection prevention*. As estratégias de busca utilizadas para as principais bases de dados encontram-se nos Apêndices 1 a 3.

Quando apropriado, utilizou-se filtros de busca específicos para ensaios clínicos randomizados (p.ex.: *Cochrane Highly Sensitive Search Strategies for identifying Randomized Trials* para a base de dados *Medline via Pubmed*).

As buscas foram conduzidas em outubro de 2015, e atualizadas em 30 de Dezembro de 2016, incluindo as seguintes bases de dados:

a) *Cochrane Central Register of Controlled Trials (CENTRAL, 2016, issue 12)*;
b) *Medline (via Pubmed)1946-2016*;
c) *Embase (Elsevier)1974-2016*;

d) *Science Citation Index Expanded and the Conference Proceedings Citation Index on Web of Science (Thomson Reuters), 1945-2016;*
e) *LILACS, 1982-2016*.

Buscou-se por estudos em andamento e/ou não publicados junto as seguintes plataformas: (a) Registro de Ensaios Clínicos da Organização Mundial da Saúde; (b) *Clinical Trials and Current Controlled and Pharmaceutical Abstracts;* (c) metaRegister of Controlled Trials; (d) OpenSigle; e (e) Google Scholar.

Foram enviadas mensagens eletrônicas para 82 destinatários, incluindo autores e experts no assunto e indústrias farmacêuticas e de alimentos contendo probióticos, com vistas a identificar potenciais estudos não publicados ou em andamento, bem como literatura cinzenta (tipo de informação ou resultados da investigação produzida pelas organizações, fora dos canais de publicação e distribuição comerciais ou acadêmicos) e trabalhos apresentados em anais de eventos científicos.Todas as listas de referências dos ECR incluídos foram revisadas por dois autores (PAW e PJFVB) em busca de estudos adicionais.

4.1.3.1 Tipos de estudo

Foram incluídos nesta RS apenas ensaios clínicos randomizados. Delineamentos observacionais não foram incluídos. Nesta RS, optou-se por não incluir ECR por agrupamento (tipo "*cluster*") e delineamentos tipo cross-over.

4.1.3.2 Participantes

Foram incluídos nesta RS os ECR que recrutaram pessoas idosas (considerando o limite etário de 65 anos ou mais), independente do sexo, comorbidades ou status cognitivo e funcional. Os indivíduos idosos poderiam estar vivendo na comunidade ou em instituições de longa permanência, e poderiam estar hospitalizados no momento da admissão no estudo. Os estudos que incluíram sujeitos com diagnóstico clínico e/ou laboratorial de infecções na admissão foram excluídos, bem como os estudos cujos indivíduos tenham desenvolvido infecções nosocomiais.

Foram excluídos desta RS os ECR cujas amostras tenham sido recrutadas:

(a) em ambientes de terapia intensiva e/ou após o uso de ventilação mecânica;

(b) com pacientes imunocomprometidos;

(c) com pacientes com doença oncológica conhecida; e

(d) após intervenções cirúrgicas.

Tendo em vista que o perfil da morbidade infecciosa nestes subgrupos pode ter apresentação clínica heterogênea, característica pelo acometimento por patógenos atípicos e/ou evolução clínica mais grave e severa[58,59], sua inclusão poderia aumentar sobremaneira a heterogeneidade dos desfechos avaliados nesta RS.

Em estudos conduzidos com indivíduos idosos e não-idosos, sempre que possível, primou-se por incluir apenas os dados pertinentes ao subgrupo com idade igual ou superior a 65 anos. Quando os dados disponibilizados nos textos completos e material suplementar eram insuficientes para garantir a inclusão apenas dos dados do subgrupo idoso, tentou-se obter dados não publicados com os autores de cada estudo. Considerou-se que os dados estavam indisponíveis quando os autores não retornaram as tentativas de contato após três oportunidades distintas e distantes pelo menos trinta dias umas das outras. Para os ECR cujos dados pertinentes ao grupo idoso foram considerados indisponíveis, esta limitação será destacada na interpretação dos resultados.

4.1.3.3 Intervenções

Incluiu-se nesta RS apenas os ECR que utilizaram cepa(s) identificada(s) de probióticos, em dose eficaz (pelo menos 10^7 unidades-formadoras de colônia/ grama). Estudos cuja descrição da intervenção não tenha possibilitado a identificação de cepa(s) probiótica(s) específica(s) (iogurte genérico, por exemplo) não foram considerados.

As intervenções poderiam incluir cepas únicas ou múltiplas, em uma ou múltiplas doses diárias, por qualquer duração de tratamento. Tanto preparações comercialmente disponíveis quanto produtos manipulados foram aceitos. Os probióticos poderiam ser dispensados sozinhos ou em combinação com prebióticos. Análise de subgrupos foi realizada para identificar se o consumo da associação de probióticos/prebióticos (também chamada de simbióticos) diferia do consumo isolado de probióticos em relação aos desfechos considerados nesta RS.

Foram considerados elegíveis apenas os ECR que utilizaram placebo como comparador. Estudos que empregaram intervenções farmacológicas ou nenhuma intervenção como comparador não foram considerados porque sua inclusão não permitiria a análise adequada da efetividade da intervenção. Estudos que tenham administrado outras drogas previamente à randomização (por exemplo, antibióticos) foram considerados elegíveis para esta RS.

4.1.3.4 Desfechos

Foram incluídos nesta RS os estudos que tenham avaliado e descrito pelo menos um dos seguintes:

a) Desfechos primários:

- ocorrência de infecção: o número de pacientes que desenvolveu um episódio de infecção para cada grupo, sobre o total de participantes de cada grupo;
- ocorrência de eventos adversos: o número de eventos adversos para cada grupo, sobre o total de participantes de cada grupo;
- mortalidade: o número de óbitos em cada grupo, sobre o total de participantes de cada grupo;
- qualidade de vida: o efeito sobre a percepção de qualidade de vida entre os grupos (independentemente do tipo de instrumento empregado), sobre o total de participantes de cada grupo;

b) Desfechos secundários:

- duração dos episódios de infecção: duração média dos episódios de infecção entre os participantes que desenvolveram infecção em cada grupo, sobre o total de participantes de cada grupo;
- ocorrência de visitas a unidades de urgência e emergência (UUE): o número de pacientes que desenvolveu infecção e que procurou atendimento em UUE em cada grupo, sobre o total de participantes de cada grupo;
- ocorrência do uso de antibióticos: o número de pacientes que desenvolveu infecção e que recebeu a prescrição de antibióticos em cada grupo, sobre o total de participantes de cada grupo;
- duração do tratamento com antibióticos: duração média da prescrição de antibióticos entre os pacientes que desenvolveu infecção em cada grupo, sobre o total de participantes de cada grupo;

- ocorrência de hospitalização: número de pacientes que desenvolveu infecção e foi admitido em unidade hospitalar para o tratamento desta condição em cada grupo, sobre o total de participantes de cada grupo;
- duração da hospitalização: duração média da hospitalização entre os pacientes que desenvolveram infecção e demandaram admissão hospitalar para seu tratamento em cada grupo, sobre o total de participantes de cada grupo.

4.1.4 Extração e análise de dados

4.1.4.1 Seleção dos estudos

A relação completa de todas as citações identificadas mediante a aplicação das estratégias de busca nas múltiplas bases de dados foi inserida no software gerenciador de referências *EndNote* versão X7 (*Thomsom Reuters, 2013*). Após a exclusão das duplicatas entre as diferentes bases pelo software, o autor principal (PAW) checou manualmente novamente todas as citações; posteriormente, dois autores (PAW e PJFVB) avaliaram, de modo independente, a relação de estudos potencialmente elegíveis. Os títulos e resumos de cada citação foram individualmente revisados e codificados, mediante a aplicação dos critérios de elegibilidade, em formulário próprio, como: *Elegível*, *Inconclusivo* ou *Não Elegível*. Os autores de manuscritos cujos título e resumo não fornecessem informações suficientes para a codificação foram contatados, com vistas a disponibilizar essas informações. Diferenças entre as avaliações dos revisores pertinentes ao processo de codificação foram resolvidas por consenso.

Dois autores (PAW e PJFVB) analisaram de modo independente os textos completos de todas as citações codificadas como *Elegível* e *Inconclusivo*, com vistas a confirmar a elegibilidade (critérios de inclusão para delineamento, participantes, intervenções e desfechos). Quando a análise dos textos completos (principalmente nos estudos codificados como “Inconclusivo”) revelou o não cumprimento dos critérios de elegibilidade (ou a presença de critérios de exclusão), registrou-se em formulário próprio as razões para a remoção destes ECR do escopo desta RS. Todos os estudos foram minuciosamente avaliados para permitir a inclusão de apenas um manuscrito no caso de múltiplas publicações do mesmo estudo. Diferenças entre os revisores na aplicação dos critérios de elegibilidade foram resolvidos por consenso.

4.1.4.2 Extração dos dados e realização da metanálise

Os dados dos ECR incluídos nesta RS foram extraídos de modo independente por dois revisores (PAW e PJFVB) utilizando um formulário padronizado, com vistas a minimizar o risco de erros no processo de extração, e assegurar que os mesmos dados fossem registrados para cada estudo. O formulário foi testado previamente pelos autores em um piloto para essa RS. Sempre que necessário, um dos revisores (PAW) contatou os autores de estudos para requisitar dados adicionais ou informações relevantes, complementares a extração dos textos completos.

Registraram-se os detalhes do delineamento, características dos participantes, caracterização das intervenções e desfechos estudados, bem como os principais resultados e conclusões e a estratégia PICO de cada ECR incluído.

Desfechos dicotômicos foram extraídos como o número de participantes que apresentou o desfecho em cada grupo e o número total de participantes nos grupos intervenção e controle. Para os desfechos contínuos, foram extraídos a média aritmética e respectivo desvio-padrão para cada grupo, em conjunto com o número total de participantes, ou extraordinariamente, a mediana e intervalo de confiança, quando disponível. Discordâncias sobre os dados extraídos por cada revisor foram resolvidos por consenso, e quando necessário, um terceiro revisor (APV) independente foi consultado.

Empregou-se o software *Review Manager versão 5.3.5* - RevMan (*Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014*) nas etapas de descrição e apresentação dos resultados da metanálise desta RS, avaliação da heterogeneidade estatística e do risco de viés.

4.1.4.3 Avaliação do risco de viés

Três revisores (PAW, PJFVB e VSN) acessaram de modo independente o risco de viés dos ECR incluídos empregando o "*The Cochrane Collaboration Risk of Bias tool*" (CCRBt).[60] Os seguintes domínios e respectivas classificações, adaptados de Hao e colaboradores[61], foram adotadas:

a) Geração da lista de randomização:

- Baixo risco de viés = adequada geração da seqüência de alocação (por exemplo, números randômicos gerados por computador, tabela de números randômicos ou similar);
- Alto risco de viés = inadequada geração da seqüência de alocação (número de prontuário, data de nascimento, mês ou dia da admissão, ou alocação gerada pelo julgamento do pesquisador, participante, por resultados de exames complementares ou disponibilidade da intervenção);
- Inconclusivo = a geração da seqüência de alocação não foi suficientemente clara ou adequadamente descrita.

b) *Segredo da alocação*:

- Baixo risco de viés = adequado sigilo da alocação (por exemplo, unidade central independente, envelopes selados não-translúcidos; ou similar)
- Alto risco de viés = inadequado sigilo da alocação (qualquer procedimento que seja evidente antes da alocação, por exemplo, alternância, utilização do registro de prontuários, datas de nascimento, tabelas abertas de números randômicos ou similar);
- Inconclusivo = descrição insuficiente do sigilo da alocação (por exemplo, não especificar ou descrever qualquer abordagem para garantir a ocultação).

c) Mascaramento dos participantes e pesquisadores:

- Baixo risco de viés = o mascaramento de ambos participantes e pesquisadores foi considerado (por exemplo, placebo idêntico a intervenção, pesquisadores não eram capazes de distinguir as intervenções nem os grupos);
- Alto risco de viés = estudos abertos;
- Inconclusivo = informação disponível insuficiente para julgar o nível de viés.

d) Mascaramento na avaliação dos desfechos:

- Baixo risco de viés = mascaramento do pesquisador responsável por acessar os resultados dos desfechos;
- Alto risco de viés = ausência de mascaramento para a detecção dos desfechos;

- Inconclusivo = informação disponível insuficiente para julgar o nível de viés.

e) Dados incompletos dos desfechos:

- Baixo risco de viés = o estudo não tinha dados de desfechos faltantes ou apresentava poucas exclusões, e uma análise de intenção-de-tratar (ITT= "*intention to treat*") foi possível;
- Alto risco de viés = havia grandes diferenças nas exclusões entre os grupos ou a taxa de exclusões foi superior a 15%, independentemente de ter sido utilizada análise de ITT;
- Inconclusivo = a taxa de exclusões foi superior a 10%, mas menor que 15%, independentemente de ter sido utilizada análise de ITT.

f) Relato seletivo:

- Baixo risco de viés = protocolo do ECR estava disponível, e os desfechos relatados não diferiram dos desfechos previstos no protocolo;
- Alto risco de viés = protocolo do ECR estava disponível, e os desfechos relatados diferiram dos desfechos previstos no protocolo (por exemplo, foram incluídos desfechos não previstos ou omitidos desfechos previstos no protocolo);
- Inconclusivo = protocolo não disponível.

g) Outros vieses:

- Baixo risco de viés = não foi percebido potencial conflito de interesses (por exemplo, ausência de financiamento e/ou apoio da indústria alimentícia e farmacêutica ou vinculação dos pesquisadores com estas);
- Alto risco de viés = percebido potencial conflito de interesses (por exemplo, financiamento e/ou apoio da indústria alimentícia e farmacêutica ou vinculação dos pesquisadores com estas)
- Inconclusivo = informação disponível insuficiente para julgar o nível de viés.

Tendo em vista que essa RS foi desenvolvida junto ao Programa de Pós-Graduação em Saúde Coletiva, optou-se por aplicar concomitantemente o "*Effective Public Health Practice Project Quality Assessment tool*" (EPHPP-QAt)[62] na avaliação da qualidade dos estudos incluídos. A ferramenta foi desenvolvida pelo núcleo de pesquisadores do Centro de Prática Baseada em Evidências da Universidade de *McMaster* (Canadá); em estudo de

análise metodológica, quando comparado ao CCRBt, a EPHPP-QAt demonstrou melhor concordância inter-avaliadores, possivelmente por mensurarem construtos diferentes.[62] Como a última foi desenvolvida com foco na Saúde Pública, o resultado de sua aplicação pode vir a fornecer informações adicionais relevantes para a aplicação do conhecimento oriundo de uma RS no campo da Saúde Coletiva.

O resultado da aplicação do instrumento supra-mencionado não será apresentado no artigo final desta tese, tendo em vista que não é um dos objetivos mais importantes deste trabalho, mas será incluído como apêndice, e será considerado na elaboração de potencial futuro manuscrito comparando suas propriedades metodológicas com as do instrumento do CCRBt, particularmente no campo da Saúde Coletiva.

O viés de publicação nesta RS foi avaliado por meio de análise qualitativa do gráfico de funil (*funnel plot*) se mais de dez estudos tiverem sido incluídos na metanálise do desfecho em avaliação. Razões para as assimetrias serão discutidas, se eventualmente encontradas.

4.1.4.4 Unidade de análise e síntese e análise dos dados

A unidade de análise considerada nesta RS foi a individual. Nos ECR com mais de dois braços de intervenção (probióticos em dose eficaz, usando concentrações em doses baixas *versus* elevadas, por exemplo), foram incluídos apenas os dados das intervenções de dose mais elevada.

Para desfechos dicotômicos os resultados foram apresentados como razão de risco(RR), com intervalo de confiança de 95%. Para dados contínuos, os resultados foram apresentados como diferença média, se os desfechos foram medidos utilizando os mesmos métodos entre os ECR. Empregamos a diferença de médias padronizada para combinar os dados do mesmo desfecho que foram mensurados utilizando-se de métodos diferentes entres os ECR.

Utilizou-se o modelo de efeito fixo sempre que seus pressupostos tiverem sido aceitos, ou seja, desde que o efeito de interesse tenha sido o mesmo em todos os estudos, e que as diferenças observadas entre eles se dê apenas a erros amostrais (variabilidade). Caso contrário, adotou-se o modelo de efeitos randômicos (ou aleatórios). Quando a medida de efeito foi proveniente de dados dicotômicos, a estimação do efeito foi calculada

com o recurso do método de *Mantel-Haenszel*. Nos desfechos contínuos, utilizou-se o método do inverso da variância.

4.1.4.5 Dados faltantes

Sempre que necessário, o revisor principal (PAW) contatou os autores dos estudos primários para complementar informações insuficientes ou dados faltantes. Utilizamos os dados de análises ITT sempre que as amostras consistiram de participantes que, uma vez randomizados, receberam ao menos uma dose da intervenção alocada e participaram de pelo menos uma avaliação de desfechos após o recrutamento.

4.1.4.6 Avaliação e investigação da heterogeneidade

A heterogeneidade estatística foi avaliada por meio de inspeção visual do gráfico de floresta (*forrest plot)*, e mediante a análise das estatísticas do teste do I^2 e do teste do chi-quadrado ($p<0.01$). Os valores do I^2 foram interpretados de acordo com as recomendações da Colaboração Cochrane:

- 0 a 40%: pode não ser importante;
- 30 a 60%: pode representar moderada heterogeneidade;
- 50 a 90%: pode representar significativa heterogeneidade;
- 75 a 100%: pode representar considerável heterogeneidade.

Foram realizadas análises de subgrupo para todos os desfechos cuja estatística do I^2 foi superior a 50%, na tentativa de identificar possíveis justificativas para a elevada heterogeneidade. Os seguintes subgrupos foram analisados, sempre que possível:

(a) idade (65 - 80 anos; 80 anos ou mais) ;

(b) ambiente do recrutamento (hospital; instituição de longa permanência; comunidade).

(c) tipo de intervenção probiótica (cepas isoladas de probióticos; probióticos associado com prebióticos; cepas de múltiplos probióticos combinados)

(d) tipo de infecção investigada (infecções gastrointestinais; infecções do trato respiratório; todas as infecções);

4.1.4.7 Análises de sensibilidade

Aplicamos análise de sensibilidade excluindo os ECR com elevado e/ou inconclusivo risco de viés para o sigilo de alocação e dados incompletos dos desfechos. Do mesmo modo, quando necessário, aplicamos esta análise nos casos de imputação de dados para explorar potenciais diferenças entre os modelos estatísticos (efeito fixo *versus* efeito randômico), desde que os pressupostos para a utilização dos dois modelos tenham sido aceitos.

4.1.4.8 Síntese de dados

Para a descrição e apresentação dos resultados da metanálise desta RS empregou-se o gráfico de floresta (*forrest plot*). Este gráfico particulariza as informações individuais dos estudos primários incluídos nesta RS, bem como os resultados, para cada desfecho da MA, sumarizando em uma única figura as informações sobre o efeito/precisão da intervenção e a contribuição de cada estudo para esta análise, quando no mínimo três estimativas estiverem disponíveis por desfecho.

4.1.4.9 Avaliação da qualidade da evidência utilizando o Sistema GRADE

Utilizou-se o Sistema GRADE (*Grading of Recommendations Assessment, Development and Evaluation*) para avaliar a qualidade da evidência gerada por esta RS. Abordamos os seguintes desfechos neste processo:

- ocorrência de infecções;
- mortalidade;
- eventos adversos;
- qualidade de vida;
- duração dos episódios de infecção.

Utilizou-se a ferramenta *GRADEprofiler versão 3.6.1 (GRADE Working Group 2004-2011)* para importar os dados do software RevMan, de modo a criar a tabela de Sumário das Evidência (*Summary of finding´s tables*). Nesta, apresentou-se um resumo do efeito da intervenção e a mensuração da qualidade da evidência para cada um dos desfechos supra-mencionados, utilizando-se das recomendações previamente publicadas para o emprego do Sistema GRADE[63–65].

O Sistema GRADE utiliza cinco critérios (limitações metodológicas, consistência do efeito; imprecisão; evidência indireta e viés de publicação) para reduzir (ou elevar) o nível da qualidade da evidência para cada desfecho. A evidência foi rebaixada em um nível para limitações "sérias" (ou em dois níveis para limitações "muito sérias"), dependendo da avaliação individual de cada critério[63–65].

A escolha da relevância dos desfechos foi orientada pela sua importância para o paciente, e não pelas informações disponíveis na literatura, conforme recomenda o Sistema GRADE. Como desfechos críticos foram selecionados mortalidade, eventos adversos e prevenção de infecções. Como desfechos importantes, duração média dos episódios de infecção.

4.1.4.10 Desvios e alterações em relação ao protocolo original

Durante o desenvolvimento desta RS e MA, algumas modificações em relação ao protocolo originalmente publicado junto a PROSPERO foram identificadas como necessárias pelos autores, ou sugeridas pela banca durante o exame geral de qualificação. Dentre estas destacam-se:

a) permitir a inclusão de estudos cujos pacientes tenham sido recrutados em ambiente hospitalar;

b) excluir os estudos cuja população tenha sido recrutada entre participantes criticamente enfermos, imunocomprometidos, portadores de neoplasia ou pacientes cirúrgicos;

c) incluir os desfechos qualidade de vida e mortalidade como desfechos primários, e não secundários.

4.2 **Publicação 2**: Protocolo da Revisão Sistemática

Nutrition Bulletin

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Effectiveness of probiotics for preventing infections in the elderly: Systematic review and meta-analysis – study protocol

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Abstract

Older persons have greater susceptibility to infections than younger adults, with generally more severe and atypical episodes. Probiotics are living microorganisms that confer a health benefit to the host when administered in sufficient quantities. Studies in older people receiving probiotic supplementation suggest a potential role of probiotics in infection prevention. We present a systematic review protocol aimed to assess the efficacy of probiotics in the prevention of community acquired infection in elderly people living either in the community or in long-term care facilities. We will include only randomised controlled trials (RCTs). We will comply with the recommendations of the Cochrane Collaboration and the principles PRISMA Statement. This protocol is registered in the PROSPERO database (CRD42014013707).

Keywords: aged, aged 65 and over, infection prevention, probiotics, systematic review

Background

Description of the condition

The proportional and absolute number of people aged over 65 years is growing at an unprecedented rate, especially in the subgroup older than 85 years (Liang & Mackowiak 2007). Previous economic evaluations have shown that older people are responsible for one of every three inpatient stays, accounting for almost $329 billion in hospital charges (Liang & Mackowiak 2007). This suggests that, by 2020, three-quarters of overall mortality in developing countries may be due to age-associated disease (Gavazzi *et al.* 2004; Liang & Mackowiak 2007).

Most deaths in developed countries elderly are due to non-communicable disease (*e.g.* cancer, heart failure, diabetes) but the impact of infectious disease is more significant in the developing world (Gavazzi & Krause 2002; Gavazzi *et al.* 2004). Almost 5% of the elderly population of Europe and the United States will die as a consequence of infectious disease, while this proportion is expected to be around 20% in Africa (WHO 2001; Videcnik Zorman *et al.* 2013).

Infections may be defined by clinical, laboratory and additional parameters, depending mainly, but not exclusively, on the type of infection, the site of infection and the patient's immune response. In older patients, infections may present with atypical manifestations: fever may be absent or blunted, complicating prompt and accurate diagnosis (Yoshikawa 1997).

Older persons have greater susceptibility to infections than do younger adults, with generally more severe and

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atypical episodes (Yoshikawa 1997; Htwe *et al.* 2007; Steens *et al.* 2014). With aging, the ability to ward off infectious agents is compromised by changes in non-adaptive immunity (immunosenescence) and the impact of chronic disease, malnutrition, medication effects, and functional deficits (Yoshikawa 1997; Strausbaugh 2001; Liang & Mackowiak 2007; Steens *et al.* 2014). Functional dysregulation of the immune system has been postulated as a plausible cause for increased susceptibility of the elderly to infectious disease (Pawelec *et al.* 2010; Dewan *et al.* 2012; Pae *et al.* 2012).

Despite the current use of effective antimicrobial agents and preventive measures, infection stands out as a major cause of morbidity and mortality in the elderly (Laurent *et al.* 2012). Studies on community acquired infections in the aged suggest that the most common site is the respiratory tract (40–43%), followed by the urinary tract (22–27%) and skin and soft tissue infections (13–15%) (Liang & Mackowiak 2007; Videcnik Zorman *et al.* 2013). Although the majority of the elderly population live in the community, long-term care residents are more often admitted to emergency departments and hospital units for infection; these infections are of greater severity and have greater associated mortality than those seen in community dwellers (Yoshikawa 1997; Videcnik Zorman *et al.* 2013; Steens *et al.* 2014).

Infections in the elderly will contribute significantly to the increase in societal and health costs in developing countries over the coming decades, an effect compounded by the aging of the population (Gavazzi *et al.* 2004). Although infection control programmes have been described, the lack of long-term impact on outcomes does not support either their widespread use or their global implementation (Gavazzi *et al.* 2004). Considering that environmental and genetic interventions are not easily implemented (let alone available in clinical practice), nutritional interventions have been recommended as promising alternatives in an attempt to reduce the impact of infectious disease in the elderly (Guillemard *et al.* 2010a, 2010b; Pae *et al.* 2012).

Unfortunately, many authors have published the positive results of improvements in laboratory parameters but failed to demonstrate benefits in health-related outcomes (Katan 2012). In the face of the pharmaceutical and food industries' massive appeals for the consumption of certain nutritional products, claiming health benefits, it is essential that clinical trials actually succeed in providing outcomes that are relevant for public health, especially for individuals in whom immune dysregulation may predispose to infectious illness.

Description of the intervention

The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations define probiotics as living microorganisms that confer a health benefit to the host when administered in sufficient quantities (WHO 2001).

Probiotics should be able to reach the digestive tract alive and to remain viable during passage through the stomach and exposure to bile (WHO 2001; Bermudez-Brito *et al.* 2012; Duncan & Flint 2013).

Several probiotics are available worldwide. The most commonly employed strains are: the *Lactobacillus* species *acidophilus, bifidum, brevis, buchneri, bulgaricus, casei paracasei* (subspecies *paracasei* and *tolerans)*, *caucasicus, coryniformis, crispatus, delbrueckii, fermentum, gasseri, GG, helveticus, johnsonii, lactis, leichmannii, plantarum, reuteri, rhamnosus*, and *salivarius;* the *Bifidobacterium* species *animalis, bifidum, breve, clausii, infantis, lactis*, and *longum;* the *Saccharomyces* species *boulardii, cerevisiae* (or *cerevisiae boulardii);* and the *Enterococcus* species *faecalis* and *faecium*.

Other species used as probiotics include: *Streptococcus mitis*, *oralis*, *rattus*, *salivarius*, *sanguis*, *thermophilus*, and *faecium*; and *Bacillus clausii*, *coagulans*, *licheniformis*, *oligonitrophilus*, *stearothermophilus*, and *subtilis*. Typical doses used in studies have been at least 10^7–10^{10} colony forming units/gram (WHO 2001; Bermudez-Brito *et al.* 2012). Probiotics can be found in commercially available preparations or in compound products.

Probiotics are available as isolated strains, multiple strains and in combination with other substances such as prebiotics (Bermudez-Brito *et al.* 2012; Duncan & Flint 2013). Prebiotics are non-digestible fibre compounds that act as a substrate for, and stimulate the growth and activity of, advantageous bacteria. They increase the number and activity of lactic acid bacteria, including the probiotic strains.

How the intervention might work

At least six major mechanisms of action have been described for probiotics: (a) modulation of the immune system; (b) inhibition of pathogen adhesion; (c) enhancement of the intestinal epithelial barrier; (d) competitive exclusion of pathogenic microorganisms; (e) increased adhesion to the intestinal mucosa; and (f) production of anti-microorganism metabolites (Bermudez-Brito *et al.* 2012).

The possible effects of probiotic consumption include the production of vitamins in the intestinal lumen and

the production of substances, such as acetate and lactate, that inhibit pathogens (WHO 2001; Bermudez-Brito *et al.* 2012; Duncan & Flint 2013). There is a significant age-associated reduction in the proportion of some physiological fecal flora species in the bowel lumen, especially *Bifidobacterium*, and the administration of substances containing probiotics may augment the presence of these flora in the stool, reducing inflammation. In addition, probiotics have the potential ability to modulate immunosenescence in the elderly. All of these effects have been studied as possible roles of probiotics in the prevention and treatment of infections in humans (Claesson *et al.* 2012).

Studies in older people receiving probiotic supplementation for 3 months identified a reduction in the average duration per episode of infection compared with placebo and a reduction in the frequency of common infectious diseases, mainly upper airway infections (Guillemard *et al.* 2010b). The use of probiotics also reduced the incidence of acute upper airway infections and reduced the use of antibiotics when compared with placebo. These results indicate the potential role of probiotics in infection prevention (Hao *et al.* 2011).

Importance of this review

As the population ages rapidly in developing countries, the impact of infectious disease in the elderly is likely to become a major public health problem (Boraschi *et al.* 2010). The economic costs related to hospitalisation and antibiotic treatment in the elderly are significant, as is the individual and familial burden (Gavazzi *et al.* 2004). Preventive interventions that may reverse or slow the process of immunosenescence and reduce the incidence of infection are perceived as necessary to reduce overall morbidity and mortality in this age group.

Previously, trials of probiotics focused on outcome variables without clinical significance (*e.g.* laboratory surrogates) making it difficult to translate their results into benefits for users. In recent years, the recommendation of adopting significant clinical outcomes (*e.g.* impact on mortality, reduction in infectious disease incidence, improvement in quality of life) has changed how researchers interpret the results of randomized controlled trials (RCTs) of probiotics (Duncan & Flint 2013).

Previous analyses have shown health benefits of the consumption of probiotics in both human and animal models (Bermudez-Brito *et al.* 2012). Some effects, however, are likely to be strain-specific, and it is unknown whether the properties of a single strain to prevent infection can be extrapolated to all probiotics (WHO 2001; Bermudez-Brito *et al.* 2012; Duncan & Flint 2013).

A previous systematic review demonstrated protective effects of probiotics and prevention of infections in children (Hao *et al.* 2011). Despite promising data from studies targeting an elderly population (Guillemard *et al.* 2010a, 2010b; Duncan & Flint 2013), the results of RCTs remain controversial in this age group (Pae *et al.* 2012). Therefore, a systematic review on this topic is important and necessary, especially given the pressure of the pharmaceutical and food industries to promote probiotics as beneficial to health promotion and alleged infection prevention in all age groups (Wachholz *et al.* 2014).

Objectives

To assess the effectiveness of probiotics in the prevention of community acquired infection in elderly people living either in the community or in long-term care facilities.

Methods

Criteria for study consideration

Studies

We will include only RCTs. Non-standard designs, such as cluster RCTs and crossover RCTs, will not be included given the risk that the effects of the interventions could result in unit-of-analysis error or could inflate standard errors (SEs). We will comply with the recommendations of the Cochrane Collaboration, in particular with the Methodological Expectations of Cochrane Intervention Reviews (MECIR) (Chandler *et al.* 2011) and the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Moher *et al.* 2015; Shamseer *et al.* 2015). This systematic review protocol is registered in the PROSPERO database, registration number CRD42014013707.

Participants

We will include studies that recruited elderly people (aged 65 years or older), regardless of sex, comorbidities, or functional or cognitive status. Individuals may live in the community or be institutionalised (long-term care units, residential care or nursing homes) and must have no clinical or laboratory signs or diag-

nosis of infection at baseline. Patients that develop nosocomial infections will be excluded.

In studies conducted with mixed populations (individuals under and over 65 years), only data relevant to the elderly will be collected. When the data related to the elderly group are not available in the full text or supplementary files, we will seek additional information from the authors. If we do not succeed in gathering this information, this bias will be highlighted in the interpretation of this systematic review results, and suggestions to improve future clinical trials on probiotics will be addressed in the conclusions.

Interventions

We will accept any intervention that involves specific and identified probiotic strains (such as *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus*, *Bacillus*) at an effective dose regimen (at least 10^7 colony forming units/gram). Interventions may involve single or multiple strains, single or multiple doses, alone or in combination with prebiotics, and any duration of treatment. Any route of administration is acceptable except spinal, and either commercially available preparations or compound products are acceptable.

Statistical analysis will be used to separately evaluate the efficacy of interventions with single and multiple strains, and of interventions with and without prebiotics.

Only placebo comparators will be considered. Studies that use other pharmacological interventions or no intervention for comparison will not be considered because these may not allow the pure assessment of the probiotics. Studies where other drugs were administered prior to randomisation will be eligible.

Outcome measures

Studies will be included if they report at least one of the clinical outcome measures.

Primary outcomes

(a) Prevention of infection: the number of patients who did not develop an infection for each treatment group against the number of participants in each group; and (b) incidence of adverse events: the number of adverse events in each treatment group against the number of participants in each group.

Secondary outcomes

(a) Prevention of emergency department visits: the number of patients (of those who developed infection) who did not seek treatment at an emergency department against the number of participants in each group; (b) prevention of antibiotic use: the number of patients who did not use antibiotics (of those who developed bacterial infection) for each treatment group against the number of participants in each group; (c) duration of antibiotic treatment: the mean duration and standard deviation (SD) of antibiotic treatment (in those who developed bacterial infections) for each treatment group against the number of participants in each group; (d) duration of episodes of infection: the mean duration and SD of infection episodes (in those who developed infections) for each treatment group against the number of participants in each group; (e) duration of hospitalisation due to infection: the mean duration and SD of hospitalisation due to infection (in those who developed infections that required hospitalisation) for each treatment group against the number of participants in each group; (f) prevention of hospitalisation for infection treatment: the number of patients who did not undergo hospitalisation for infection treatment (of those who developed infections) for each treatment group against the number of participants in each group; (g) quality of life: the effect on the perception of quality of life among the intervention groups, using generic or specific tools and scales for quality of life measurement already validated for each treatment group against the number of participants in each group; and (h) all-cause mortality.

Search methods for study identification

We will attempt to identify all relevant studies regardless of language, publishing period or publication status (*i.e.* published, unpublished, in press, ongoing). The electronic search will be conducted by the main reviewer (PAW), and searches for unpublished trials, reference lists and grey literature will be performed by three authors (PAW, PJFVB, VSN). We will use specially designed and tested search filters (*e.g.* Cochrane Highly Sensitive Search Strategies for identifying randomised trials in MEDLINE) where appropriate.

Electronic searches

We will search the following databases for relevant studies, using the search terms detailed in Appendix 1: (a) the Cochrane Central Register of Controlled Trials (CENTRAL), published in *The Cochrane Library*; (b) MEDLINE – Medical Literature Analysis and Retrieval System Online (Pubmed); (c) EMBASE – Excerpta Medica dataBASE (OVID); (d) ISI Web of Knowledge;

and (e) Latin American and Caribbean Health Science Literature (LILACS). We will also search the WHO clinical trial registry platform and the metaRegister of Controlled Trials (mRCT) for ongoing trials.

Other resources

We will check the reference lists of all studies identified by the above methods for other potentially relevant studies. In addition, we will search for information on unpublished studies in the WHO International Clinical Trials Registry Platform, Clinical Trials and Current Controlled Trials and Pharmaceutical Abstracts (1970 to present). We will also ask experts and contact pharmaceutical companies for further information on grey literature, and we will use OpenSIGLE (1993 to present) and Google Scholar to look for unpublished trials presented at scientific conferences.

Data collection and analysis

Study selection

For all studies identified, two authors (PAW, VSN) will independently screen and review the titles and abstracts. They will code studies as 'retrieve' (eligible or potentially eligible/unclear) or 'do not retrieve', using a standardised form. Whenever necessary, the chief investigators of selected trials will be contacted (through electronic contacts and/or addresses listed in the manuscripts, or through their institutions) to provide additional relevant information if eligibility is unclear. Disputes regarding the retrieval of a study will be resolved by a third author (PJFVB).

For the studies coded 'retrieve' we will obtain the full-text publication. The authors previously mentioned (PAW, VSN) will independently screen and identify studies for inclusion, recording exclusion reasons for the ineligible studies. Each trial's reports will be scrutinised to ensure that only a single publication from each study is included. Disagreements about selection and inclusion/exclusion of studies will be resolved by consensus, and an independent reviewer (PJFVB) will be consulted if disagreement persists.

Reference management software will be used to manage the records retrieved and to highlight duplications among the different databases. Any article in a language other than English will be translated. All data collected in the selection process will be used to complete a PRISMA flow chart and a table of excluded studies (Moher *et al.* 2015).

Data extraction and management

Data will be extracted independently by two reviewers (PAW, VSN), using a standardised form to minimise the risk of error and to ensure that the same data are recorded for each study. This form will be pretested in a pilot. Whenever necessary, the chief investigators of selected trials will be contacted to provide additional relevant information.

Details of the study design, participant characteristics, intervention characteristics, primary and secondary outcomes (with randomised and analysed numbers in each treatment group), adverse effects and methodological quality (randomisation, blinding, allocation concealment, and assessment of bias risk) will be extracted from each of the included studies. The Quality Assessment Tool for Quantitative Studies, developed by the *Effective Public Health Practice Project* (*EPHPP*), will be used as part of our data extraction form.

Dichotomous outcomes will be extracted as the number of participants who experienced the outcome in each group, with the total sample size for both intervention and comparator groups. For continuous outcomes, we will extract arithmetic means and SDs for each treatment group, together with the number of participants in each group. If only SE is reported, SD will be calculated, as long as sample size is known, using SE = SD / square root of the sample size. Disagreements about extracted data will be resolved by consensus, and an independent reviewer (PJFVB) will be consulted if disagreement persists.

Assessment of bias risk

To assess the risk of bias in the included studies, three review authors (PAW, PJFVB, APV) will independently use The Cochrane Collaboration's Risk of Bias tool (Higgins *et al.* 2011). Accordingly, the following domains will be assessed: (a) random sequence generation; (b) allocation concealment; (c) blinding of staff, researchers and participants; (d) similarity of baseline outcome measurements; (e) similarity of baseline characteristics; (d) blinding of outcome assessment; (e) incomplete outcome data; (f) selective reporting; and (g) other bias. Each of these criteria will be explicitly judged by assigning: (a) low risk of bias; (b) high risk of bias; or (c) unclear risk of bias (where unclear relates to the lack of precise information or uncertainty over the potential for bias).

Whenever necessary, the chief investigators of selected trials will be contacted to provide additional relevant information. Disagreements between the authors regarding the risk of bias will be resolved by

consensus, and a fourth reviewer (APV) will be consulted if disagreement persists.

Measures of treatment effect

Dichotomous data: results will be presented as the risk ratio with 95% confidence intervals. Continuous data: results will be presented as the mean difference, if outcomes are measured by the same method between trials. We will use the standardised mean difference to combine trials that measure the same outcome using different methods.

Unit of analysis issues

The appropriate unit of analysis will be by individual patient, rather than by hospital or centre. In studies with multiple intervention groups, we will include only those groups that meet the eligibility criteria. If more than one intervention group of interest is identified, the analysis will be divided into pair-wise comparisons.

Missing data

Whenever necessary, we will contact the chief investigators of clinical trials with missing data or unclear information (*e.g.* unclear risk of bias). We will use intention-to-treat (ITT) analysis wherein the ITT sample consists of participants who, when randomised, took at least one dose of the assigned study intervention and participated in at least one post-baseline assessment.

Assessment of heterogeneity

Heterogeneity will be assessed by visual inspection of the forest plot and by using both the I-squared statistic and the Chi-squared test (with statistical significance defined as $P < 0.10$). I-squared values will be interpreted according to the current Cochrane Collaboration guidance (Higgins *et al.* 2011) as follows: (a) 0–40%: might not be important; (b) 30–60%: may represent moderate heterogeneity; (c) 50–90%: may represent substantial heterogeneity; or (d) 75–100%: considerable heterogeneity. If the data are sufficiently similar, we will perform quantitative synthesis by means of meta-analysis. If appropriate, we will perform meta-regression.

Assessment of reporting biases

We will search for protocols of included trials through PubMed and trial registries, whenever possible. We will contact study authors to attempt to obtain the complete data or the reasons for the non-reporting of specific outcomes. If there are sufficient numbers of trials (at least ten), we will construct a funnel plot to look for evidence of publication bias.

Data synthesis

We will summarise data using the Cochrane Collaboration's Review Manager statistical software version 5.3 (2014). When significant clinical or methodological heterogeneity exists, we will use a random-effects model. Otherwise, we will choose the fixed-effect model for data synthesis. We will pool data, using the relative risk for dichotomous outcomes and the median difference for continuous outcomes. For random-effects meta-analysis, we will use the DerSimonian and Laird method. Where the data are too heterogeneous for combining effect sizes in a meaningful or valid manner, we will present the results of individual studies using a narrative approach in the main text of the review.

Subgroup analysis and investigation of heterogeneity

We will identify heterogeneity statistically, through forest plot analysis, using the I-squared statistic to determine if clinical heterogeneity might pose a problem in combining trial results. We will consider an I-squared value higher than 50% to have high heterogeneity, and a value of less than 25% will be considered to have low heterogeneity. If possible, we intend to perform subgroup analysis to consider the following: (a) age [young elderly (65–79 years) and very elderly (80 years or older)]; (b) coexistence environment (institutionalised vs. community-dwelling); (c) type of probiotic (probiotics combinations vs. individual probiotics strains); and (d) type of infection.

Sensitivity analysis

Where appropriate, we will perform sensitivity analysis, excluding trials with high or unclear risk of bias and trials without full-text information. Sensitivity analysis will also be performed to explore the differences between different models (fixed-effect vs. random-effect) for pooling data where there is heterogeneity. We will also perform this analysis for trials with imputed data.

Risk of bias table

For the risk of bias table, the following outcomes will be considered: (a) prevention of infection; (b) survival; (c) prevention of hospitalisation for infection treatment;

(d) prevention of emergency department visit; (e) prevention of antibiotic use; (f) quality of life; and (g) incidence of adverse events.

Rating quality of evidence

We will analyze the overall strength of the evidence for each outcome using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool (Guyatt *et al.* 2008; Balshem *et al.* 2011).

For each outcome that includes pooled data, we will analyse the following study limitations: inconsistency, imprecision, indirectness of evidence, risk of bias, and potential publication bias. When appropriate, we will downgrade the evidence from 'high quality' by one level to 'serious', or by two levels to 'very serious'.

Using GRADEprofiler software (GRADEPRO), we will create 'summary of findings' tables, with the intention to provide outcome specific information concerning the overall quality of evidence from studies included and the magnitude of effect of the interventions examined (Purgato *et al.* 2014).

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Author contributions

Patrick Alexander Wachholz: conception and design, data analysis and interpretation, final version revision. Vânia dos Santos Nunes: conception and design, data analysis and interpretation, final version revision. Adriana Polachini do Valle: data analysis and interpretation, final version revision. Hélio Rubens de Carvalho Nunes: data analysis and interpretation, final version revision. Paulo José F. Villas Boas: conception and design, data analysis and interpretation, final version revision.

Conflict of interest

The authors have no conflict of interest to disclose.

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Appendix 1

Appendix 1. Preliminary search strategy for MEDLINE and EMBASE

Search set	MEDLINE	EMBASE
1	('Probiotics/administration and dosage'[Mesh] OR 'Probiotics/therapeutic use'[Mesh])	*probiotic agent / dt [Drug Therapy]
2	'lactobacillus'[Mesh]) OR 'lactococcus'[Mesh]) OR 'bifidobacterium'[Mesh]) OR 'enterococcus'[Mesh]) OR 'streptococcus'[Mesh]) OR 'saccharomyces'[Mesh]	'Lactobacillus'[Emtree]) OR 'Lactococcus'[Emtree]) OR 'Bifidobacterium'[Emtree]) OR 'Enterococcus'[Emtree]) OR 'Streptococcus'[Emtree]) OR 'Saccharomyces'[Emtree]
3	lactobacill*[tiab]	Lactobacill*ti, ab
4	lactococc*[tiab]	Lactococc*ti, ab
5	bifidobacter*[tiab]	Bifidobacter*ti, ab
6	enterococc*[tiab]	Enterococc*ti, ab
7	streptococc*[tiab]	Streptococc*ti, ab
8	saccharomyces [tiab]	Saccharomyces ti, ab
9	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10	'older' OR 'senior*' OR 'elderly' OR 'geriatric' [tiab]	'Older' OR 'senior*' OR 'elderly' OR 'geriatric' ti,ab
11	('Aged'[Mesh]) OR 'Aged, 80 and over'[Mesh]	Aged [Emtree]
12	10 OR 11	10 OR 11
13	9 AND 12	9 AND 12

Search terms used in combination with the search strategy for retrieving trials developed by the Cochrane Collaboration.
This is the preliminary search strategy for MEDLINE and EMBASE. It will be adapted for other electronic databases. We will use a sensitised search strategy, developed by the Cochrane Collaboration, for identifying randomised controlled trials. All search strategies will be reported in full in the final version of the review.

5. Resultados, Discussão e Conclusões

Resenha de manuscrito: ***"Effectiveness of probiotics for preventing infections in older person: Systematic Review and Meta-Analysis****"*

ABSTRACT

Background: infectious diseases in older persons are associated with higher mortality rates, and prevention is generally confined to adherence to immunization and hygiene measures. Probiotics were hypothesized as an alternative approach to infection prevention.

Objectives: this Systematic Review and Meta-Analysis aims to assess the effectiveness of probiotics in the prevention of infections in older adults when compared to placebo, as well as the safety of this intervention.

Methods: randomised placebo-controlled trials of probiotics for prevention of infection in older person were identified in MedLine, Embase, CENTRAL, Web of Science and Lilacs, on 30 December 2016. Efficacy outcomes were occurrence of infection, quality of life, mortality and mean duration of infection per episode. Safety outcomes were adverse events. Data were analysed using relative risk ratios and mean differences with 95% confidence intervals. Relative risk ratios were pooled where more than three estimates were available.

Results: fifteen reports were included (total n= 5,916 participants, with a mean age of 75.21 years). The effect of probiotics was not significantly different from that noted to placebo on the occurrence of infection, mortality, adverse events or mean duration of infection episodes (RR 0.90, 95%CI 0.76—1.08; RR 1.01, 95% CI 0.91—1.12; RR 1.09, 95% CI 0.70—1.72; MD -0.35, 95% CI -1.57—0.87, respectively).

Conclusion: in older patients, current moderate quality of evidence do not support the use of probiotics for prevention of infection. There is a low quality of evidence that suggests that the mean duration of an infection episode is not affected by probiotics.

PROSPERO: CRD42014013707

Key-words: probiotics; aged; aged, 80 and over; infection

BACKGROUND

Infectious diseases (ID) in older persons are more frequent, usually more severe, and undoubtedly associated with higher mortality rates and functional impairment than in younger adults[1]. More than 21 million infection-related hospitalizations were estimated among US older patients between 1990-2002, with an upward trend in hospitalization rates related in part to increasing admissions of patients older than 75 years old[2]. The burden of ID on older adults visits to US emergency departments in 2011-2012 was found to be higher than that for myocardial infarction and congestive heart failure combined[3].

The prevention of ID in older person is generally confined to adherence to immunization, sanitation and hygiene measures. Probiotics were hypothesized as an alternative approach to infection prevention[4]. Probiotics are live microorganisms, usually sold as dietary supplements, which when administered in adequate amounts confer a health benefit on the host[5]. They are thought to create a favourable gut environment and to support a healthy digestive tract and a healthier immune system[5].

Randomised clinical trials (RCT) and systematic reviews (SR) have consistently found benefits related to probiotic consumption on the occurrence, duration and severity of ID in paediatric patients, when compared to placebo[4,6,7]. Although age-related immunosenescence is unmistakably associated with higher ID rates in older patients[8], the effectiveness of probiotics on the prevention of infection in older age groups remains controversial[9–15].

The primary purpose of this SR was to assess the effectiveness of probiotics in the prevention of infections in older adults when compared to placebo, as well as the safety of this intervention.

METHODS

Criteria for considering studies for this review

The protocol of this review was registered on PROSPERO database (CRD42014013707), and it was previously published[16]. We complied with the recommendations of the *Methodological Expectations of Cochrane Interventions Reviews*, and with the principles of the *PRISMA* statement.

We included only RCT; observational designs and non-standard experimental designs (such as cluster and crossover RCTs) were not considered.

The studies must have recruited people aged 65 years or older, regardless of sex, comorbidities, or functional or cognitive status. In studies conducted with mixed populations (individuals under and over 65 years), we tried to include only data relevant to the older group, whenever possible. Individuals might be living in the community or in long-term care units, and must had no clinical or laboratory signs nor diagnosis of infection at baseline. Participants could be hospitalized at recruitment, but patients that developed nosocomial infections were excluded. When data were not available in the full text or supplementary files, we contacted authors for additional information, whenever possible. As the pattern and severity of ID in subgroups of older persons that are critically ill and immunosupressed or experiencing oncologic and postoperative conditions may be substantially different and heterogeneous, we excluded these studies from this analysis.

We accepted any intervention that involved specific and identified probiotic strains at an effective dose regimen(at least 10^7 colony-forming units/gram)[16]. Interventions could involve single or multiple strains, using single or multiple doses, alone or in combination with prebiotics, at any duration of treatment. Either commercially available preparations or compound products were acceptable. Only placebo comparators were considered; studies that used other pharmacological interventions or no intervention for comparison were not considered. Studies where other drugs were administered prior to randomisation were considered eligible. For studies with two or more active arms, we included only data relevant to the highest intervention dose.

Studies were included if they reported at least one of the following outcome measures:

- **Primary Outcomes**: occurrence of infection; incidence of adverse events; mortality; quality of life.
- **Secondary Outcomes**: duration of episodes of infection; occurrence of visits to emergency departments; occurrence of antibiotic use; duration of antibiotic treatment; occurrence of hospitalization; duration of hospitalization.

Search methods for identification of studies

We aimed to identify all relevant studies, regardless of language, publishing period, or publication status. The electronic search was conducted by the first author(PAW), and searches for unpublished trials, reference lists and grey literature were performed by two authors(PAW, PJFVB). We used specially designed and tested search filters for identifying RCT in Medline[17]. The full

search strategy was composed of the following terms(and its main synonyms): aged; aged, 80 and over; probiotics; *lactobacillus*; *lactococcus*; *bifidobacterium*; *enterococcus*; *streptococcus*; *saccharomyces*; and infection prevention, as shown in the appendices. We searched the following databases on 30 December 2016:

1. Cochrane Central Register of Controlled Trials(CENTRAL, 2016, issue 12),
2. Medline(Pubmed),1946-onwards,
3. EMBASE(Elsevier),1974-onwards,
4. Science Citation Index Expanded and the Conference Proceedings Citation Index on Web of Science(Thomson Reuters),1945-onwards,
5. Latin American and Caribbean Health Science(LILACS),1982-onwards,

We also searched the WHO International Clinical Trial Registry Platform, Clinical Trials and the metaRegister of Controlled Trials for ongoing studies. In addition, we asked experts and contacted pharmaceutical and probiotic companies for further information on grey literature and unpublished RCT; we also searched for additional studies on conference proceedings and Google Scholar.

A reference management software was used to manage the records retrieved; duplications among the different databases were highlighted by the software, and checked manually by the first author(PAW). Two authors(PAW, PJFVB) independently assessed all titles and abstracts, obtained full texts for potentially relevant studies and applied the eligibility criteria. Any discrepancies regarding eligibility was resolved by consensus. If more than one publication reported data from the same participants, the most detailed study was used.

Data were included as stated in the published papers whenever possible; where data were insufficiently reported, we contacted the authors for clarification. Data from eligible studies were extracted by two independent reviewers (PAW, PJFVB) using a pre-tested extraction form. We created a study flow diagram using PRISMA model(Figure 1), to map out the numbers of records identified, included and excluded; a table with excluded studies and reasons is available in the appendices.

We extracted the details of each included RCT; they are available in the appendices, and summarized at Table 1. Three reviewers(PAW, PJFVB, VSN) independently assessed methodological information using the Cochrane´s tool for Risk of bias assessment[17], adopting the criteria outlined in the appendices. Disagreements were resolved by consensus.

Data collection and analysis

The appropriate unit of analysis was by individual patient. For all outcomes, we carried out analyses on an intention-to-treat basis, including all randomised participants to each group, in the group they were allocated. We assessed statistical heterogeneity using I^2 statistic, and interpreted it according to the Cochrane Handbook[17], considering it substantial if I^2 was greater than 50%. If there were 10 or more studies in the MA, we investigated reporting biases using funnel plots; reasons for asymmetry were considered if they were noted.

We used the Review Manager software(RevMan 5.3®) to calculate summary statistics using a random-effects model. For continuous data we provided the mean difference(MD) if outcomes were measured in the same way among trials, standardized mean difference(SMD) if not. For dichotomous data, we presented results as summary risk ratio(RR) with 95% confidence intervals(CI). Where substantial heterogeneity was present, we considered potential explanations including subgroups analysis, and conducted a sensitivity analyses based on the risk of bias, in which we excluded studies with the highest level of bias or unclear bias for allocation concealment and completeness of outcome data.

We considered subgroup analyses for the following, whenever possible:

(1) age (65-80y; older than 80 years);

(2) coexistence environment (institutionalized, hospitalized, community-dwelling);

(3) type of probiotics(combinations of probiotics, probiotics plus prebiotics, individual strains);

(4) type of infection.

We produced a 'Summary of findings table' to report the intervention effect and a measure of the certainty of the evidence for each outcome using the GRADE approach, including a narrative description, when necessary.

The GRADE approach uses five considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of the body of evidence for each outcome. The evidence was downgraded from 'high' by one level for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, indirectness of evidence, serious inconsistency, imprecision of effect estimates or potential publication bias.

We applied minor changes to the original protocol[16], with the intention of improving the sensitivity of results, and to adapt them to GRADE standards: a) we allow the inclusion of trials whose patients have been recruited in a hospital setting; b) we excluded studies whose population has been recruited from critically ill, immunocompromised, oncologic or surgical patients; c) we included quality of life and mortality as primary outcomes, rather than secondary outcomes.

RESULTS

Results of the search, Excluded and Included studies

The search retrieved 5,036 studies(Figure 1), among which 15 reports were included(Table 1). We excluded 56 RCTs(more details in the appendices). The main reason for exclusions was being an oncologic or post-operative patient(17 studies). All included studies were completed and published; authors of three ongoing studies did not answer our contacts and were not included.

The included studies enrolled 5,916 participants(intervention=2,959;control=2,957), from nine countries (Table 1), with a mean age of 75.2 years and a median follow-up time of 319 days (204.75-631.50). Eight studies recruited inpatients[11,13,15,18–22] and investigated the effect of probiotics in the prevention of *Clostridium difficile* diarrhoea (CDD); in three RCTs patients were admitted from long-term care institutions (evaluating respiratory tract infections (RTI)[9] or all infectious disease (AID)[10,23]), and in four reports participants were living in the community (RTI[12,24,25] and AID[14], respectively).

Eight reports enrolled only older patients [9–14,23,24], corresponding to 86.3% of the participants. The mean age in four out of the six remaining studies were higher than 65 years old [18–21]; one author provided non-published data on older person[25], and in one report the randomisation schedule was generated using a rule that included age at entry (<65vs≥65y)[22].

Two ECR[10,11] recruited solely functional dependent older participants(n=96); one of them[11] exclusively enrolled bed-ridden dementia patients. Three studies[9,14,23] enrolled independent older patients(n=1,843), while most studies did not registered functional capacity nor cognitive status. No study used probiotics associated with prebiotics.

Risk of bias in included studies

Six studies did not clearly report the random sequence generation nor the allocation concealment[10,11,15,18,23,24]. Blinding of outcome assessment was insufficiently reported in nine RCT[9–11,14,18,21–23,25], and five studies were judged high risk of bias for incompleteness outcome data due to a high rate of exclusions and/or attrition[9,10,19,20,23].

There was not enough information to fully assess the potential for selective reporting bias for most of included trials, so we judged them as being at an unclear risk. In the five studies where the protocol was available[10,13,20,20,25], all outcomes were reported as intended (as shown in the appendices). Most studies[9–12,14,18,19,21–23,25]were funded, granted or supported by probiotic industries.

Effect of interventions

See **Summary of findings** (Table 2) for main comparisons.

- *Prevention of infection:* The effect of probiotics on the occurrence of infection was not significantly different from that noted to placebo (13 trials, 5,820 participants: RR 0.90, 95%CI 0.76—1.08; I^2=24%; *Figure 2*). When only trials that enrolled older person were included, there was still no evidence of a significant effect (9 trials, 5,225 participants, 735 events: RR 0.92, 95%CI 0.77—1.09; I^2=27%). The subgroups analysis did not change the heterogeneity; the sensitivity analysis did not change the outcome result (RR 0.92, 95%CI 0.76—1.12).
- *Adverse events:* There was no significant difference between probiotics and placebo concerning adverse events (RR 1.01, 95% CI 0.91—1.12; I^2=0%). Data were available for 5.310 participants from nine trials, for a total of 1.098 events (probiotics 554; placebo 544). The most common adverse events reported were related to gastrointestinal disorders: constipation, abdominal pain and cramping, flatulence, nausea and gas bloating, with almost none cases of serious adverse events related directly related to probiotic use.
- *Mortality:* The effect of probiotics on general mortality was not significantly different from that noted to placebo (5 trials, 669 participants, 77 events: RR 1.09, 95% CI 0.70—1.72; I^2=5%). It is important to highlight that mortality was described as an outcome by a very small number of studies, representing only 13.2% of the total population analyzed in this review.
- *Quality of life (QoL):*Three[13,14,24] trials described QoL as an outcome, but only two of them[13,24] provided data for comparison analysis. Data were available for 3,136 participants. As QoL was measured using different scales, we used the SMD (SMD -0.09, 95% CI -0.99— 0.81, I^2=97%).
- *Mean duration of infection per episode:* Probiotics were no significantly different from placebo in reference to mean duration of ID episodes (7 trials, 1,406 participants, MD -0.35,

95% CI -1.57—0.87, I^2=86%). We did not find differences between groups of types probiotics, settings and types of ID during subgroups analyses to examine heterogeneity; insufficient data limited our analysis by age group. Sensitivity analysis did not significantly change the estimate of effect.

- For additional information and graphs related to above mentioned outcomes, please see the appendices. Unfortunately, the included studies did not provide sufficient data for description of the other secondary outcomes foreseen in the protocol of this SR[16].

DISCUSSION

Summary of main findings

This systematic review of probiotics for prevention of ID in older person found 15 completed RCT, including three larger trials (more than 500 subjects), enrolling 5,916 participants. Whilst five studies did not provide their data related to older participants, the mean age of their samples were over 65 years, and they accounted for less than 15% of the total participants of this SR.

Participants were recruited mainly from long-term care institutions and community settings, or during short-term hospital admissions, and were mostly represented by independent older patients. Primary studies mainly investigated the effect of probiotics on the preventions of respiratory tract infections, *Clostridium difficile* diarrhoea and on a set of common ID. Despite the high clinical heterogeneity among the primary studies, it is important to highlight that ID in older person is a complex entity: probably no single intervention would result in a direct effect of a binary outcome. Factors like seasonal changes in contact, social structure, vaccination, nutrition, frailty, immunosenescence, and setting may enhance susceptibility to infection[26].

Overall, when compared to placebo, probiotics did not significantly reduce the occurrence of ID in older person, with a moderate quality of evidence. Of note, we found a consistent effect (that did with change on sensitivity and subgroup analysis) and a narrow confidence interval, that did not cross damage or benefit thresholds. Probiotics also did not seem to reduce the mean duration of an infection episode (low quality of evidence).

Although poorly described, the effect of probiotics on general mortality did not seem to differ from placebo (very low quality of evidence). This SR did not find differences between probiotics and

placebo regarding adverse events, with moderate quality of evidence. The QoL was described by only two studies, which showed opposite results, what may justify the high heterogeneity, despite the large sample analysed in this outcome. Despite its importance, QoL remains a rather neglected outcome in studies conducted with older patients.

There are very few studies evaluating the prevalence and budge impact of the consumption of probiotics; an American study with 1.976,167 discharges found that probiotics were used in 51,723 of hospitalizations in 2012[27]. Probiotics were previously found to be better than placebo in reducing the mean duration of RTI[4]; to reduce the risk of developing CDD by 59.5% (especially among hospitalized patients)[28]; and were considered a cornerstone for the prevention of some infections in paediatric patients[6]. However, RCT of probiotics for infection prevention in older persons consistently found controversial results. In accordance with, a SR recently found no evidence of benefit of probiotics in reducing the incidence of CDD in older person[29].

Limitations of the review:

We had no access to primary data on older person from five trials; this information might have changed some endpoints. Due to the same reason, we were unable to do subgroup analysis with age groups (65-80y; >80y). We cannot make any assumptions about long-term safety about probiotics in older person, neither about their effect on critically ill older patients.

Despite our persistent contacts, no author provided us information on ongoing trials. The clinical heterogeneity of the interventions and infections studied may have contributed to the lack of statistical significance, so fixed-effect models were not possible. The follow-up period was extremely variable in each study, and the information related to vaccination and routine hygiene measures were lacking in most trials, and may have influenced the ID outcomes.

Implications for practice and research:

On average, older adults have from two to five episodes of mild infections per year, including upper RTI, flu, digestive and urinary tract infections[1]. The use of over-the-counter medications with the intention to prevent an ID is trivial, but costs, safety and the risk of drug interactions exist, and may be a concern for public health, due to the high incidence and recurrence rates of ID on this age group.

New research agenda should focus on specific strains, and their relation of dosing and timing to potential benefit. In the same way, they should include clinically relevant efficacy outcomes instead of laboratory surrogates, and focus on better description of safety outcomes.

CONCLUSION

There is no simple solution for the problem related to infectious diseases in older patients. Unique infection-control interventions may be probably not realistic. Current moderate quality of evidence do not support the use of probiotics for prevention of infection. There is a low quality of evidence that suggests that the mean duration of an infection episode is not affected by probiotics.

Conflict of interest: None to declare

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Key points

- *The impact of infectious diseases on older person extends beyond the clinical, economic and social domains;*
- *The greater susceptibility of the older person to infections may be associated with immunosenescence, and few interventions have shown to be effective in their prevention in older person;*
- *Probiotics seem to have some interesting advantages when compared to other interventions, particularly related to good tolerability and safety profile, with a low prevalence of adverse events, but their effectiveness in older people are controversial.*

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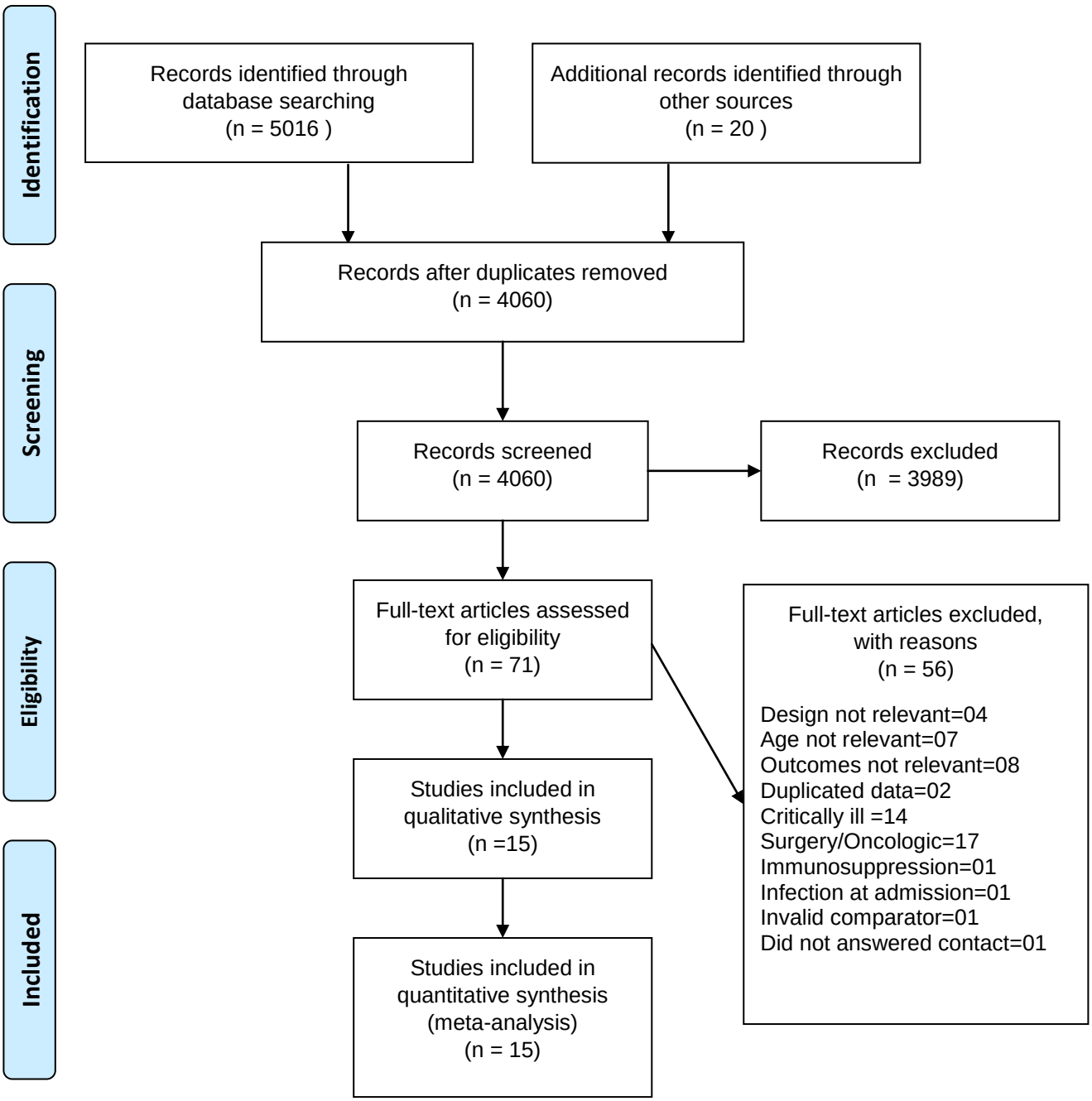

Figure 1. Study flow diagram.

Table 1. Main characteristics of included studies.

	Study design	Country and duration	P.I.C.O.	Main findings	Other relevant information
Allen 2013	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial	United Kingdom December 1, 2008 to May 30, 2012	- Elderly inpatients exposed to antibiotics in the preceding 7 days; - Multi-strain probiotic; - Placebo; - Occurrence of AAD and CDD	CDD was as uncommon cause of AAD and occurred in 0.8% participants in the probiotic group and 1.2% in placebo group (RR 0.71; 95% CI 0.34-1.47; p=0.35). Frequency of serious AE was much the same in the two groups, none attributed to participation in the trial.	A modified intention-to-treat analysis was applied (as well as PPA), excluding a small number of participants who withdrew shortly after randomisation, did not receive the interventions, and did not have follow-up data. CDD was expected to occur in 4% of placebo group, but it occurred in only 0.99% of all participants. Funders/support: National Institute for Health Research, UK
Auinguer 2013	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial	Germany October 2010 to May 2011	- Healthy adults with recurring common colds; -Single probiotic strain; - Placebo; -Incidence of common cold	Probiotic reduced the number of symptomatic common cold infections by 25% as compared to placebo (p=0.041). Data on elderly subgroup was provided by authors and there was no difference between groups in this age group.	Authors did not provided information on adverse events on the 16 participants older than 65 years old. Funders/support: The study was funded by Leiber GmbH (Germany). The authors are employed by a contract research organization.
Beausoleil 2007	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	Canada September 2003 to May 2004	- Adult inpatients who were anticipated to take at least 3 days of any systemic antibiotic; - Multi-strain probiotic; - Placebo; - Occurrence of AAD/CDD	Daily administration of probiotics was safe and effective in the prevention of AAD; 1 patient in probiotic group and 7 in placebo group developed CDD (essay performed in only 2 patients in intervention group and in 13 in placebo group).	Funders/support: Supported and granted provided by Bio-L+ International Inc.
Fujita 2013	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial	Japan November 30, 2009 to June 30, 2010.	- Elderly users of day care facilities; - Single strain probiotic; - Placebo; - Occurrence of URTI	The number of persons diagnosed with an acute URTIs was much the same in both groups. Mean duration of infection per event was found to be shorter in probiotic group (3.71 x 5.40 days).	Subjects diagnosed with an acute URTI, to compare the mean duration of infection per diagnosed subject and mean duration of infection per infection event between groups. Funders/support: Supported by Yakult Honsha Co Ltd. Authors claimed that the company had no involvement in the research.
Fukushima 2007	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial	Japan Winter seasons 2002-2003	- Bed-ridden inpatients(>70 years) with dementia and using total EN; -Multi-strain probiotic; - Placebo; - Incidence of infection	The percentage of days with infections significantly decreased (15.4% v. 5.7%) in the probiotic group comparing the run-in period with intervention period; the reduction was larger than that of the control group (p=0.047)	Funders/support: Grant from the Applied Science Board for Lactic Acid Bacteria (Tokyo). There is no Conflict of Interest statement, but the main authors were employes of Nestlé Business Group and Research Centre and the intervention drink patent belongs to Nestlé.
Guillemard 2010	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial	France November 2, 2006 to May 4, 2007	- Non-institutionalized elderly older than 70y; - Multi-strain probiotic; - Placebo; - Occurrence of common infectious diseases	Probiotic intervention significantly reduced the average duration per episode of CID (6.5 v. 8d; p=0.008) and the cumulative duration of CID (7 v.8d; p=0.009). The cumulative number of CID was not different between groups.	The authors assumed that an average number of 1.5 events would be observed in the control group, with an estimated 15% relative decrease in intervention group. Funders/support: The study was sponsored and funded by Danone Research.
Hickson 2007	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial	England November 2002 to January 2005	- Inpatients aged 50y and over who were prescribed antibiotics; - Multi-strain probiotic;	Nine/53 patients in control group had CDD v. none/56 in intervention group(p=0.001; absolute risk	Funders/support: Healthcare Foundation and Hammersmith Hospital Trustees research committee and Danone.

			- Placebo; - Incidence of AAD and CDD	reduction was 17%, NNT=6.	
Lewis 1998	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	England Duration not clearly stated	- Elderly inpatients who were prescribed antibiotics; - Single strain probiotic; - Placebo; - Occurrence of AAD and CDD	There was no difference in the incidence of CDD between groups (I=5/33; C=3/36).	Funders/support: United Bristol Health Care
Mañé 2011	Randomised, double blind, placebo controlled, multicentre, three-arm parallel pilot trial	Spain Winter 2006 and Spring 2007	- Institutionalized elderly; - Multi-strain probiotic; - Placebo; - Infection occurrence and survival rates	Incidence of infection showed a significant trend to be lower in the high probiotic dose group. Mortality was greater in placebo vs. both probiotic groups.	Funders/support: Funded by CARINSAm ACC10 (Catalan Government) and PROFIT-CDTI (Spanish Government)
Nagata 2016	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	Japan September 2009-2010	- Institutionalized elderly; - Single strain probiotic; - Placebo; - Infections and fever occurrence	Long-term consumption of probiotic may be useful for decreasing risk of infection among long-term care elderly (number of days with fever/2 weeks after 6th month consumption was 1.1vs.2.5 at intervention and placebo group, respectively).	The authors considered the mean number of days with fever as a surrogate to infection diagnosis. Funders/support: Supported by Yakult Honsha Co Ltd
Pozzoni 2012	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	Italy April 2009 to July 2010	- Inpatients aged 50y and over who had not yet started receiving antibiotics or who were prescribed antibiotics < 48h; - Single strain probiotic; - Placebo; - Incidence of AAD and CDD	Five cases of CDD occurred, 2 in the placebo group (2%) and 3 in the probiotic group (2.8%); the probiotic was not effective in preventing AAD/CDD.	Funders/support: Ad hoc hospital fund for independent research
Safdar 2008	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	United States November 2003 to June 2005	- Adult inpatients aged 18 years and older who were prescribed antibiotics for at least 72h; - Single strain probitic; - Placebo; - Incidence of AAD and CDD	Probiotic intervention was well tolerated, without major AE; CDD occurred in only one patient in placebo group; of 7 patients tested (I=3; P=4; p=0.27)	Funders/support: Supported by Lifeline probiotic industry
Shinkai 2013	Randomised, double blind, placebo controlled, single centre, three-arm parallel trial	Japan March to July 2010	- Elderly living in the community; - Single strain probiotic; - Placebo; - Occurrence of common cold	The accumulated incidence rate of CC was 47.3% on placebo group and 29% on high-dose intervention group (p=0.012); QOL (SF-36) was higher on intervention groups (p=0.016)	Funders/support: No specific grant nor funding declared.
Thomas 2001	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial	United States July 1998 to October 1999	- Adult inpatients aged 18 years and older who were prescribed antibiotics for at least 72h; - Single strain probitic; - Placebo; - Incidence of AAD and CDD	Probiotics did not reduced the rate of occurrence of AAD. (p=0.93). Too few patients had positive diagnosis of CDD to assess between group differences.	Patients included were older than 18 years old. Although aged participants were included, mean age in both groups were younger than 65 years old; the authors did not mention if there were differences according to age (groups were stratified as younger/older than 65 years at enrollment). Funders/support :Supported by a grant from CoAgra Foods Inc
Van Puyenbroeck 2012	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial	Belgium Winter 2007-2008	- Institutionalized elderly; -Single strain probiotic; - Placebo; - Occurrence of RTI	Probiotic had no statistically or clinically significant effect on protection against respiratory symptoms.	Duration of respiratory symptoms was reported as the number of days with respiratory symptoms/effective number of days of participation multiplied by 100; the occurrence of infection was reported as participants with

at least one day of RTI symptoms.

Funders/support: Supported by Yakult Honsha Co Ltd

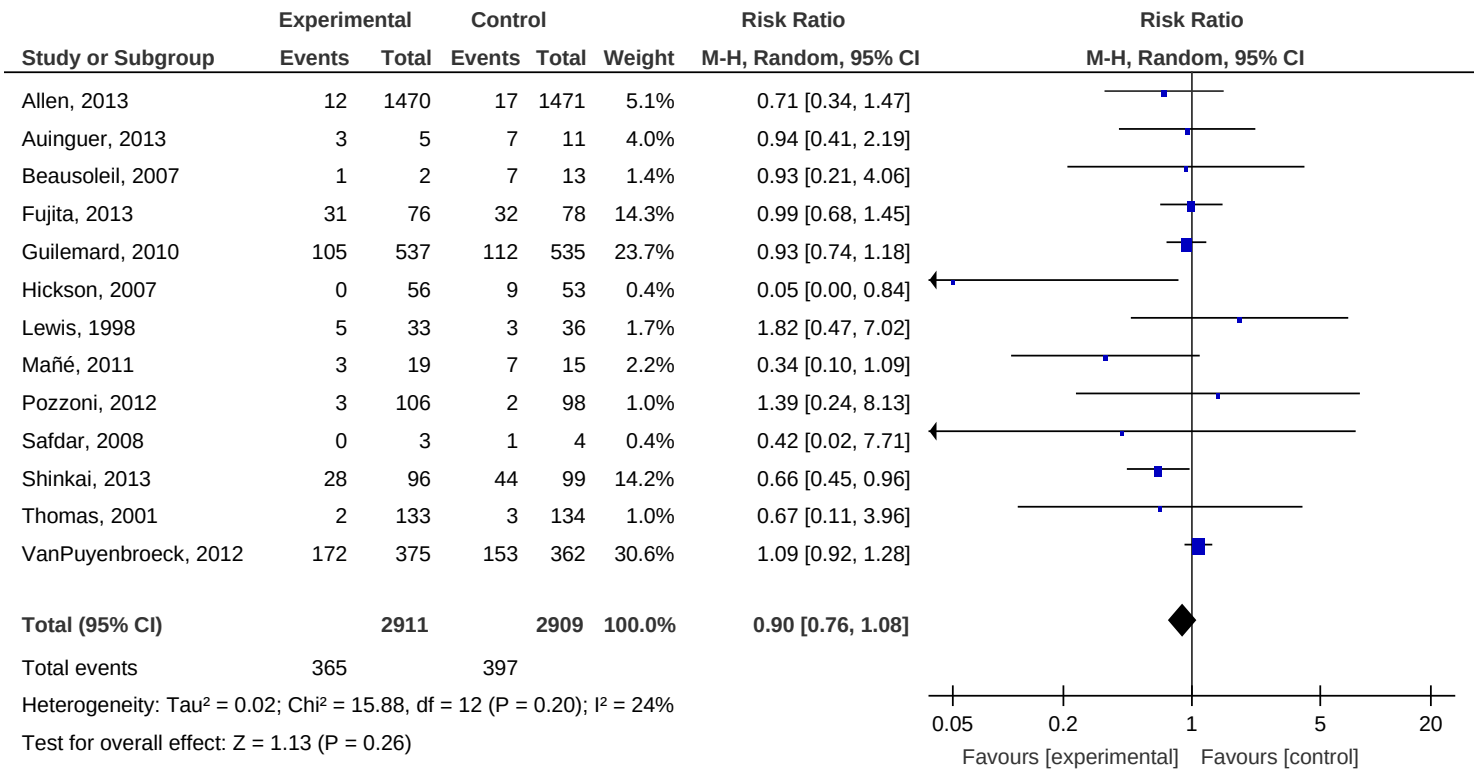

Figure 2. Comparison between intervention (probiotics) versus placebo for the Primary Outcome ***Occurrence of infection*** (alphabetically ordered studies).

Table 2. Summary of findings tables

Probiotics for infection prevention in older person

Patient or population: patients with infection prevention in older person
Settings:
Intervention: Probiotics

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Control	Corresponding risk Probiotics	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
Prevention of infection clinical and/or laboratory signs of infection (for CDD) Follow-up: 92-1276 days	**Study population**		**RR 0.9** (0.76 to 1.08)	5820 (13 studies)	⊕⊕⊕⊖ **moderate**[1]	
	136 per 1000	**123 per 1000** (104 to 147)				
	Moderate					
	250 per 1000	**225 per 1000** (190 to 270)				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Five out of 13 studies analyzing the outcome "infection prevention" were considered as having high risk of bias for incomplete outcome data, and another three were judged as unclear for the same criterion. Although some of these studies were composed of small samples, one of them compromises more than 30% of the sample weight. Nevertheless, sensitivity analysis excluding this five studies did not change significantly the overall effect and confidence intervals.

Are probiotics safe in preventing infections in older person when compared to placebo?

Patient or population: patients with infection prevention in older persons
Settings:
Intervention: probiotics

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Control	Corresponding risk Probiotics	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
Adverse events clinical reports Follow-up: 92-1276 days	**Study population**		**RR 1.01** (0.91 to 1.12)	5310 (9 studies)	⊕⊕⊕⊖ **moderate**[1,2]	
	206 per 1000	**208 per 1000** (187 to 231)				
	Moderate					
	193 per 1000	**195 per 1000** (176 to 216)				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Safety evaluations of probiotics should consider "pathogenicity, infectivity, virulence, toxicity, metabolic activity and intrinsic properties of the microbes". Uncompleteness of outcome data affected trials that emcompasses important weight in the poof of this outcome analysis.
[2] The authors of this review found that most studies did not addressed adverse events as an outcome, only used nonspecific safety statements that "the intervention was well tolerated".

Does the use of probiotic for infection prevention in older person affects general mortality rates, when compared to placebo?

Patient or population: older person for infection prevention
Settings:
Intervention: probiotic

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Control	Corresponding risk Probiotic	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
Mortality clinical reports Follow-up: 92-1276 days	**Study population**		**RR 1.09** (0.7 to 1.72)	669 (5 studies)	⊕⊕⊖⊖ **low**[1,2]	
	110 per 1000	**120 per 1000** (77 to 189)				
	Moderate					
	122 per 1000	**133 per 1000** (85 to 210)				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Despite reasonable evidence that probably the effect of probiotic consumption in mortality risk is similar to that observed in placebo, only five out of the 15 included studies described this as a primary endpoint.
[2] Although the confidence interval was not too large, the sample size for this outcome, as weel as the number os events, were too small, and did not met the optimum size of information (OSI).

Does the use of probiotic affect the mean duration of an infection episode in older persons, when compared to placebo?

Patient or population: patients with duration of an infection episode in older persons
Settings:
Intervention: probiotics
Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk Placebo	Corresponding risk Probiotics	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
Mean duration of infection per episode Follow-up: 92-1276 days		The mean mean duration of infection per episode in the intervention groups was **0.35 lower** (1.57 lower to 0.87 higher)		1406 (7 studies)	⊕⊕⊖⊖ **low**[1,2]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Incompleteness of outcome data was judged as high risk to high number of trials; the concealment of the allocation was also judged unclear to a high number of studies.
[2] The estimate effects showed different magnitudes, the confidence intervals presented small ou no overlap, with high heterogeneity.

Supplementary file 1 - Main search strategies (30 December, 2016)

MEDLINE (via PUBMED) search strategy

#1	**"Aged"[Mesh] OR "Aged, 80 and over"[Mesh] OR (older) OR (senior*) OR (elderly) OR (geriatric) OR (Centenarians) OR (Nonagenarians) OR (Octogenarians) OR (Oldest Old)**
#2	**"Probiotics/administration and dosage"[Mesh] OR "Probiotics/therapeutic use"[Mesh] OR "lactobacillus"[Mesh] OR "lactococcus"[Mesh] OR "bifidobacterium"[Mesh] OR "enterococcus"[Mesh] OR "streptococcus"[Mesh] OR "saccharomyces"[Mesh] OR (lactobacill*) OR (lactococc*) OR (bifidobacter*) OR (enterococc*) OR (streptococc*) OR (saccharomyces)**
#3	**"Infection/prevention and control"[Mesh] OR (infection prevention) OR (infection control)**
#4	**randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [pt] OR evaluation studies as topic [mh] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])**
	(((((("Aged"[Mesh] OR "Aged, 80 and over"[Mesh] OR (older) OR (senior*) OR (elderly) OR (geriatric) OR (Centenarians) OR

	(Nonagenarians) OR (Octogenarians) OR (Oldest Old)))) AND (("Probiotics/administration and dosage"[Mesh] OR "Probiotics/therapeutic use"[Mesh] OR "lactobacillus"[Mesh] OR "lactococcus"[Mesh] OR "bifidobacterium"[Mesh] OR "enterococcus"[Mesh] OR "streptococcus"[Mesh] OR "saccharomyces"[Mesh] OR (lactobacill*) OR (lactococc*) OR (bifidobacter*) OR (enterococc*) OR (streptococc*) OR (saccharomyces)))) AND (("Infection/prevention and control"[Mesh] OR (infection prevention) OR (infection control)))) AND (((randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [pt] OR evaluation studies as topic [mh] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])))

EMBASE search strategy

#1	('elderly'/exp) OR ('very elderly'/exp) OR ('centenarians'/exp) OR ('nonagenarians'/exp) OR ('octogenarians'/exp) OR ('older') OR ('senior') OR ('elderly') OR ('geriatric') OR ('centenarians') OR ('nonagenarians') OR ('octogenarians') OR ('oldest old') OR ('very elderly')
#2	('probiotic agent'/exp) OR ('probiotic agent') OR ('lactobacillus'/exp) OR ('lactobacillus') OR ('lactococcus'/exp) OR ('lactococcus') OR ('bifidobacterium'/exp) OR ('bifidobacterium') OR ('enterococcus'/exp) OR ('enterococcus') OR ('streptococcus'/exp) OR ('streptococcus') OR (lactococc*) OR (bifidobacter*) OR (enterococc*) OR (streptococc*) OR ('saccharomyces'/exp) OR ('saccharomyces')
#3	('infection prevention'/exp) OR ('infection prevention') OR ('infection control'/exp) OR ('infection control')
	[embase]/lim NOT [medline]/lim
	#1 AND #2 AND #3

LILACS search strategy

	MH:"aged" OR MH: "aged,80 and over" OR (older) OR (senior) OR (elderly) OR (centenarian*) OR (nonagenarian*) OR (octogenarian*) OR (oldest old) OR MH:M01.060.116.100.080 OR MH:01.060.116.100
	MH:"Probiotics/administration & dosage" OR MH:"Probiotics/therapeutic use" OR (probiotic$) OR MH:"lactobacillus" OR MH:"bifidobacterium" OR MH:"enterococcus" OR MH:"streptococcus" OR MH:"saccharomyces" OR tw:(lactobacil*) OR tw:(lactococc*) OR tw:(bifidobacter*) OR tw:(enterococc*) OR tw:(streptococc*) OR tw:(saccharomyces) OR MH:J02.500.456.500 ORMH:B03.510.460.400.410.475.475 OR MH:B03.510.550.450.475 OR MH: B03.510.400.800.500 OR MH:B03.510.550.737.500 OR MH:B03.510.024.100 OR MH:B03.510.460.400.400.049.100 OR MH:B03.510.550.250.250 OR MH:B03.510.400.800.872 OR MH:B03.510.550.737.872 OR MH:B01.300.107.795.785 OR MH:B01.300.930.705
	MH:"Infection control" OR MH:"infection" OR (infection prevention) OR (infection) OR MH:N06.850.780.200.450 OR MH:C01.539

Supplementary file 2

Table 1. Characteristics of excluded studies *[ordered by study ID]*

Study	Reasons for exclusion
Allen, 2013[1]	Duplicated data
Allen, 2013[2]	Duplicated data
Anderson, 2004[3]	Exclusion criteria: surgical inpatients
Askerov, 2011[4]	Authors did not respond to three distinct contact attempts
Barraud, 2010[5]	Exclusion criteria: recruitment of critically ill patients
Bleichner, 1997[6]	Exclusion criteria: recruitment of critically ill patients
d´Ettore, 2015[7]	Exclusion criteria: recruitment of immunosupressed patients
Ehrhardt, 2016[8]	Age of participants not relevant
Flatley, 2015[9]	Study design not relevant
Forestier, 2008[10]	Exclusion criteria: recruitment of critically ill patients
Gerritsen, 2016[11]	Study design not relevant
Giamarellos, 2009[12]	Exclusion criteria: recruitment of critically ill patients
Graubaum, 2012[13]	Age of participants not relevant
Hilton, 1992[14]	Age of participants not relevant
Ishikawa, 2015[15]	Outcomes not relevant
Jain, 2004[16]	Exclusion criteria: recruitment of critically ill patients
Kanazawa, 2005[17]	Exclusion criteria: surgical inpatients
Kenna, 2016[18]	Exclusion criteria: recruitment of critically ill patients
Kwon, 2015[19]	Exclusion criteria: recruitment of critically ill patients
Knight, 2009[20]	Exclusion criteria: recruitment of critically ill patients
Kotzampassi, 2015[21]	Exclusion criteria: surgical inpatients
Liu, 2011[22]	Exclusion criteria: surgical inpatients
Liu, 2013[23]	Exclusion criteria: surgical inpatients
Liu, 2015[24]	Exclusion criteria: surgical inpatients
Makino, 2010[25]	Study design not relevant
Mego, 2015[26]	Exclusion criteria: surgical inpatients
Merenstein, 2015[27]	Outcomes not relevant
Morita, 2016[28]	Age of participants not relevant; comparison not valid
Morrow, 2010[29]	Exclusion criteria: recruitment of critically ill patients
Murina, 2014[30]	Age of participants not relevant
Nomura, 2007[31]	Exclusion criteria: surgical inpatients
Okazaki, 2013[32]	Exclusion criteria: surgical inpatients
Oláh, 2002[33]	Exclusion criteria: surgical inpatients
Oláh, 2005[34]	Exclusion criteria: surgical inpatients
Oláh, 2007[35]	Exclusion criteria: surgical inpatients
Przemska-Kosicka, 2016[36]	Outcomes not relevant
Rayes, 2002[37]	Exclusion criteria: surgical inpatients
Rayes, 2005[38]	Exclusion criteria: surgical inpatients
Rayes, 2007[39]	Exclusion criteria: surgical inpatients
Rongrungruang, 2015[40]	Exclusion criteria: recruitment of critically ill patients
Roos, 2011[41]	Outcomes not relevant
Salomão, 2016[42]	Exclusion criteria: infection diagnosis at recruitment
Selinger, 2013[43]	Age of participants not relevant
Smith, 2016[44]	Outcomes not relevant
Sommacal, 2015[45]	Exclusion criteria: surgical inpatients
Sugawara, 2006[46]	Exclusion criteria: surgical inpatients
Sullivan, 2002[47]	Outcomes not relevant

Tanaka, 2012[48]	Exclusion criteria: surgical inpatients
Tubelius, 2005[49]	Age of participants not relevant
Turchet, 2003[50]	Comparison not valid
Valdes-Varella, 2016[51]	Study design not relevant
Wang, 2015[52]	Outcomes not relevant
Wright, 2015[53]	Outcomes not relevant
Yang, 2016[54]	Exclusion criteria: surgical inpatients
Zhang, 2010[55]	Exclusion criteria: surgical inpatients
Zeng, 2016[56]	Exclusion criteria: recruitment of critically ill patients

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Supplementary file 3

Characteristics and description of included studies

Allen, 2013

Study design	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial
Country	United Kingdon
Randomisation	Eligible patients were allocated sequentially by research nurses in a 1:1 ratio to the two groups (placebo or microbial preparation) of the study, according to a computer-generated random sequence, stratified by centre and using blocks of variable size. The allocation sequence was generated by the independent statistician and not available to any member of the research team until databases had been completed and locked.
Blinding	Patients, study staff, and specimen and data analysts were masked to assignment. Copies of the allocation sequence were not held at the recruiting centres.
Duration	Dec 1, 2008 to May 30, 2012
Exclusions post-randomisation	14 (Intervention=12; Control=2)
Losses to follow-up	23 (Intervention=13; Control=10)
Participants	Inpatients aged 65 years or older (mean age = 77.2 years), exposed to one or more oral or intravenous antibiotics in the preceding 7 days (or about to start antibiotic treatment at recruitment), representative of those admitted to National Health Service (NHS) and similar secondary care institutions in UK. Total number of participants: 2981 (Intervention=1493; Control=1488); none imbalances at baseline
Intervention	The microbial preparation was a lyophilized powder in a vegetarian capsule containing 6 × 10(10) live bacteria: two strains of *Lactobacillus acidophilus* (CUL60, National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30157; and CUL21, NCIMB 30156) and two strains of bifidobacterium (*Bifidobacterium bifidum* CUL20, NCIMB 30153; and *B lactis* CUL34, NCIMB 30172). The dose was one capsule per day for 21 days, with food and, when possible, between antibiotic doses.
Infections investigated	*Clostridium difficile* infection
Outcomes reported	1) occurrence of CDD within 12 weeks of recruitment; 2) Severity and duration of CDD; 3)abdominal symptoms; 4) serious adverse events; 5) duration of hospital stay; 6) Quality of life
Power and assumed risk estimate	80% to detect a 50% reduction in CDD active group (estimating that CDD would occur in 4% of patients allocated to the placebo group)
Other relevant information	A modified intention-to-treat analysis was applied (as well as PPA), excluding a small number of participants who withdrew shortly after randomisation, did not receive the interventions, and did not have follow-

	up data. The occurrence of AAD was almost half of what was expected (10.6% of all patients, from estimated 20% of participants allocated to placebo). CDD was expected to occur in 4% of placebo group, but it occurred in only 0.99% of all participants.
Protocol	ISRCTN70017204
Funders/Support	National Institute for Health Research, UK

AUINGUER, 2013

Study design	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial
Country	Germany
Randomisation	The randomisation code was created by an independent statistician prior to study chart. The random numbers in blocks of 4 were randomised in a 1:1 ratio to the two study groups.
Blinding	Patients and study staff were masked to intervention. Symptoms of infection were documented by the participants in a diary and confirmed by an investigator during the episode visit on the 5th day of each episode.
Duration	October 2010 - May 2011
Exclusions post-randomisation	2
Losses to follow-up	0
Participants	Healthy subjects aged 18-70 years with recurrent common colds (at least three common cold infections within the last 6 months prior to admission). Data on the 16 participants aged 65 years and older were provided by authors. Total number of participants: 162 (Intervention=Control=81); None imbalances declared, but there was no information on individual history of infections in the last year, nor about vaccination status.
Intervention	The intervention product contained 900mg of yeast beta-glucan (Saccharomyces cerevisiae) $10x10^6$ CFU, two capsules per day, which were consumed at breakfast and supper.
Infections investigated	Common cold infection
Outcomes reported	1) occurrence of common cold episodes; 2) Severity of common cold episodes; 3) Adverse events; 4) duration of infection
Power and assumed risk estimate	Power of 80% and significance level of 5% (two-sided) were used to estimate sample size, with no description of assumption of reduction on incidence of infection with the intervention.
Other relevant information	Authors did not provided information on adverse events on the 16 participants older than 65 years old.
Protocol	ISRCTN16094368
Funders/Support	The study was funded by Leiber GmbH (Germany). The authors are

	employed by a contract research organization.

BEAUSOLEIL, 2007

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	Canada
Randomisation	Not clearly described
Blinding	Participants and investigators were blinded to intervention
Duration	September 2003 to May 2004
Exclusions post-randomisation	0
Losses to follow-up	0
Participants	Hospitalized patients (mean age: Intervention=68.8; Control=72.9) who were anticipated to take at least three days of any systemic antibiotic. Prophylaxis with the study drug or placebo began within the first 48 h of antibiotic treatment. Total number of participants: 89 (Intervention= 44; Control=45); none imbalances declared
Intervention	The intervention drink contained *Lactobacillus acidophilus* CL 1285 + *Lactobacillus casei*, at least $50x10^9$ CFU once daily for the duration of the study.
Infections investigated	*Clostridium difficile* infection
Outcomes reported	1) Occurrence of CDAD; 2) Duration of hospitalisation; 3) Adverse effects
Power and assumed risk estimate	Power of 80% to detect a significant difference (15% reduction), assuming AAD incidence of 30% in placebo group
Other relevant information	
Protocol	Not available
Funders/Support	Supported and granted provided by Bio-L+ International Inc

FUJITA, 2013

Study design	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial
Country	Japan
Randomisation	Block randomisation was implemented in single blocks with block size 4, stratified by facility and sex, with a central enrollment system.
Blinding	Allocation was performed independently from researchers by the data center allocation coordinator, who also retained the allocation list until observations were complete. The container, external appearance, flavor, and others characteristics of the placebo drink were indistinguishable from the fermented milk containing LcS. Following manufacture, allocation was performed under blind conditions by personnel in charge

	of allocation.
Duration	November 2009 to June 30, 2010.
Exclusions post-randomisation	14
Losses to follow-up	20 (hospital admissions 10; discontinued use of day care 5; other reasons 5)
Participants	Elderly population (aged 65 years or older, mean age 83.2 years), users of four day care facilities located around Tokyo. Total number of participants: 154 (Intervention=76; Control=78); none imbalances declared, but there was no information on individual history of infections in the last year, nor about vaccination status.
Intervention	The probiotic strain used in this study was Lactobacillus casei strain Shirota (LcS), using (4×10^{10} colony-forming units). The dose was one test drink (80ml) per day during the observation period.
Infections investigated	Upper respiratory tract infections
Outcomes reported	1) occurrence of URTI event; 2) symptom score; 3) duration of infection per subject; 4) duration of infection per episode of infection
Power and assumed risk estimate	An estimated sample size of 71 persons per group was used to achieve a statistical power of 70% and rejection region of 5% from 1-sided test
Other relevant information	Subjects diagnosed with an acute URTI, to compare the mean duration of infection per diagnosed subject and mean duration of infection per infection event between groups
Protocol	Not available
Funders/Support	Supported by Yakult Honsha Co Ltd. Authors claimed that the company had no involvement in the research.

FUKUSHIMA, 2007

Study design	Randomised, double-blind, placebo controlled, multicentre, two-arm parallel trial
Country	Japan
Randomisation	Not clearly described
Blinding	The control preparation was similar to intervention and administered in blind condition for participants. It was not clearly stated if investigators and staff were masked to interventions or outcome assessment.
Duration	Winter season 2002-2003
Exclusions post-randomisation	0
Losses to follow-up	0
Participants	Elderly volunteers from bed-ridden inpatients over 70 years old (mean age: Intervention=84.4; Control=84.8), suffering from dysphasia with dementia, and fed using total enteral nutrition by nasogastric tube or gastrostomy. Total number of participants: 24 (Intervention=12; Control=12); none

	imbalances declared, but there was no information on individual history of infections in the last year, nor about vaccination status.
Intervention	Intervention contained the probiotic strain L. johnsonii La1 (NCC533) at 10(9) colony-forming units and a non-probiotic strain Streptococcus thermophilus at 10^8 colony-forming units/90 g. Test drinks were administered through a tube after feeding of enteral diet.
Infections investigated	Respiratory, intestinal or urinary tract infections
Outcomes reported	1) duration of infection Duration of infections was expressed in percentage of days with infections for 12 weeks.
Power and assumed risk estimate	Not described
Other relevant information	
Protocol	Not available
Funders/Support	Grant from the Applied Science Board for Lactic Acid Bacteria (Tokyo). There is no Conflict of Interest statement, but the main authors were employes of Nestlé Business Group and Research Centre and the intervention drink patent belongs to Nestlé.

GUILEMARD, 2010

Study design	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial
Country	France
Randomisation	Symmetric randomisation was carried out in blocks on a 1:1 ratio. Volunteers were assigned to study groups using an individual randomisation number (study product allocation concealed) and were included sequentially in accordance with the randomisation list, which was stratified by centre.
Blinding	The control drink were identical to the intervention drink, in order to mantain blinding, bur masking was stated just for participants; not clearly stated for investigators and staff
Duration	112 days (2 November 2006 to 4 May 2007)
Exclusions post-randomisation	0
Losses to follow-up	208 (I=107 major protocol deviations; C=101 major protocol deviations)
Participants	Free-living elderly volunteers (median age I=76.0; C=76.0) in 125 centres distributed in twenty-five departments in France. Individuals of at least 70 years of age who were free-living (not residing in an institution), with a Gerontological Autonomy Iso-resource Group (AGGIR) score between 5 and 6, vaccination against the influenza virus at least 14 d before inclusion; a mini-mental state score of at least 24; a BMI between 17 and 25 kg/m2 (bounds included); compliance with a dietary restriction during

	the 2 weeks preceding the product consumption phase and throughout the study.
	Total number of participants: 1072 (I=537; C=535); none imbalances at baseline
Intervention	Intervention contained 10^{10} colony-forming units (CFU)/100 g of the probiotic strain *L. casei* DN-114 001 (international reference CNCM I-1518, also named *Lactobacillus paracasei subsp. paracasei*, combined with a symbiosis of two cultures commonly used in yoghurt, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (at least 10^9 CFU/100 g for the whole symbiosis). The dose was two bottles of 100g/d, one at breakfast and one at dinner, preferably with an interval of at least 8 hours)
Infections investigated	Common infectious diseases (respiratory and gastrointestinal)
Outcomes reported	1) Cumulative number of all CID; 2) occurrence of CID; 3) Duration of CID (cumulative and average per episode); 4) time span to the first occurrence of CID; 5) severity of CID; 6) occurrence or duration of medications (prescribed or auto-administered)
Power and assumed risk estimate	80% power to detect a decrease of 15% on infection incidence, assuming an average number of 1.5 events in the control group
Other relevant information	The authors assumed that an average number of 1.5 events would be observed in the control group, with an estimated 15% relative decrease in intervention group.
Protocol	Not available
Funders/Support	The study was sponsored and funded by Danone Research

HICKSON, 2007

Study design	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial
Country	England
Randomisation	An independent statistician generated the random allocation sequence, which was stratified for hospital, sex, and two age groups (50-69 and ≥70). The sequence was given to the pharmacy on each site.
Blinding	Patients and researchers were blind to the study drink as they did not see the bottle the drink came in. Nursing staff dispensed the drinks and were instructed to pour 100 ml into a cup for the patient; they were not told which bottle contained which drink.
Duration	November 2002 to January 2005
Exclusions post-randomisation	0
Losses to follow-up	22 (I=12; C=10)
Participants	Patients were recruited mainly from orthopaedic, medical, and care of

	the elderly wards, and included inpatients aged over 50 who (mean age: I=73.7; C=73.9) were prescribed antibiotics (single or multiple antibiotics, oral or intravenous) and were able to take food and drink orally. Total number of participants: 135 (I=69; C=66); none imbalances declared at baseline
Intervention	Probiotic yoghurt drink containing *Lactobacillus casei* DN-114 001 (*L. casei imunitass*) (1.0×10^8 colony forming units/ml), *S. thermophilus* (1.0×10^8 cfu/ml), and L bulgaricus (1.0×10^7 cfu/ml). The dose was 100g (97ml) twice daily half and hour before or 1-2 hours after meals.
Infections investigated	*Clostridium difficile* infection
Outcomes reported	1) occurrence of *Clostridium difficile* infection
Power and assumed risk estimate	90% power to detect an absolute difference of 20% between the proportion of AAD at control (30%) and intervention (10%)
Other relevant information	
Protocol	National Research Register N0016106821
Funders/Support	Healthcare Foundation and Hammersmith Hospital Trustees research committee and Danone.

LEWIS, 1998

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	England
Randomisation	Not clearly described
Blinding	Participants, nursing staff and personnel managing cases were masked to grouping.
Duration	Not clearly stated
Exclusions post-randomisation	0
Losses to follow-up	3
Participants	Consecutive patients over the age of 65 (mean age: I=71.81; C=70.85) admitted acutely to the general medical wards of the Bristol Royal Infirmary were invited to participate in the study if they had been prescribed antibiotics within the preceding 24 h. Total number of participants: 69 (I=33; C=36); none imbalances declared, but there was no information on individual history of infections in the last year.
Intervention	*S. boulardii* 10^8 *cfu* (113 mg twice daily) throughout the time they received antibiotics.

Infections investigated	Clostridium difficile infection
Outcomes reported	1) occurrence of C difficile infection
Power and assumed risk estimate	Not described
Other relevant information	
Protocol	Not available
Funders/Support	United Bristol Healtch Care

MAÑÉ, 2011

Study design	Randomised, double blind, placebo controlled, multicentre, three-arm parallel pilot trial
Country	Spain
Randomisation	Randomisation was performed in separate lists for each participating center.
Blinding	Participants were blind, but staff and investigators were not blind
Duration	Winter 2006 and spring 2007
Exclusions post-randomisation	10
Losses to follow-up	06
Participants	Subjects older than 65 years, institutionalized in two geriatric centers (median age: I=71; C=69). Total number of participants: 60; Intervention high dose=20; low dose=20; Control=20); none imbalances declared, but there was no information on individual history of infections in the last year, nor vaccination.
Intervention	Subjects were randomised to receive either $1x5{\cdot}10^{8}$ cfu/day of the *L. plantarum* CECT7315/7316 mixture in 20 g of powdered skimmed milk ("low probiotic dose"), 2) $5{\cdot}10^{9}$ cfu/day of the probiotic mixture in the same vehicle ("high probiotic dose"), or 3) vehicle alone (placebo). Only data regarding the high probiotic dose was used in this MA
Infections investigated	All infections
Outcomes reported	1) Infection occurrence
Power and assumed risk estimate	Not described
Other relevant information	
Protocol	Not available
Funders/Support	Funded by CARINSAm ACC10 (Catalan Government) and PROFIT-CDTI (Spanish Government)

NAGATA, 2016

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	Japan
Randomisation	Not clearly described
Blinding	Participants and staff members of the facility were blinded to intervention
Duration	September 2009-2010
Exclusions post-randomisation	16 (I=8; C=8)
Losses to follow-up	0
Participants	Residents of a special aged care facility for elderly residents (median age 84) who could not exercise on their own and who required general assistance in living. Total number of participants: 72 (Intervention=Control=36); none imbalances declared, but there was no information on individual history of infections in the last year, nor vaccination.
Intervention	The intervention drink contained Lactobacillus casei Shirota $4x10^{10}$ colony-forming-units, consumed on a daily basis, once a day after lunch.
Infections investigated	All infections
Outcomes reported	1) Mean number of days with fever; 2) Duration of use of antimicrobial agents
Power and assumed risk estimate	Not clearly described
Other relevant information	The authors considered the mean number of days with fever as a surrogate to infection diagnosis.
Protocol	UMIN000005183
Funders/Support	Supported by Yakult Honsha Co Ltd

POZZONI, 2012

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	Italy
Randomisation	Central randomisation by hospital pharmacy was performed with a computer-generated random-number table.
Blinding	The products (*S. boulardii* or placebo) were delivered to the researchers in undistinguishable containers, which were labeled only with a sequentially numbered patient identification code, regardless of the assigned intervention group. The participants, researchers, and staff contributing to the study (doctors, nurses, and microbiologists) were unaware of the treatment allocations throughout the duration of the study.

Duration	April 2009 to July 2010
Exclusions post-randomisation	0
Losses to follow-up	71
Participants	Hospitalized patients aged over 50 years (mean age 79.2), who had not yet started receiving antibiotics or who were on an antibiotic regimen for < 48 h, regardless of the number of antibiotic medications and route of administration, for the treatment of a proven or suspected infection or for prophylaxis. Total number of participants: 275 (Intervention=141; Control=134); none imbalances declared at baseline
Intervention	Probiotic capsule (Codex, Biocodex, France, distributed by Zambon, Italy) consisting of a lyophilized preparation containing 5×10^9 colony-forming units of *Saccharomyces. boulardii*, in fasting conditions (i.e., at least 2 h before meals) twice daily.
Infections investigated	*Clostridium difficile* infection
Outcomes reported	1) Occurrence of *Clostridium difficile* infection
Power and assumed risk estimate	Power of 80% to detect 50% reduction in AAD in the treated group, from a given prevalence of 37% in the placebo group; about 20% of AAD was expected to be CDAD.
Other relevant information	
Protocol	ISRCTN86623192
Funders/Support	Ad hoc hospital fund for independent research

SAFDAR, 2008

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	United States
Randomisation	A randomisation schedule was generated by the study coordinator using blocks of four. No stratification was used.
Blinding	The intervention drug and placebo were identical, stored at the inpatient pharmacy; patients and investigators were unaware of treatment assignment
Duration	November 2003 to June 2005
Exclusions post-randomisation	1
Losses to follow-up	0
Participants	Newly admitted adult inpatients (mean age: Intervention=66.56; Control=72.47) who were placed on antibiotic therapy for presumed or proven infection on admission and were expected to receive antibiotics for at least 72h.

	Total number of participants: 40 (Intervention=23; Control=17); none imbalances declared
Intervention	One capsule three times daily for 14 days after the last dose of antibiotic or matching placebo one capsule three times daily for 14 days after the last dose of antibiotic. Active capsules (Florajen_; American Lifeline, Inc., Baraboo, WI, USA) contained Lactobacillus acidophilus (20 billion CFUs per capsule) in a gelatine capsule.
Infections investigated	Clostridium difficile infection
Outcomes reported	1) Occurrence of Clostridium difficile infection; 2) Adverse effects
Power and assumed risk estimate	Not described
Other relevant information	
Protocol	Not available
Funders/Support	Supported by Lifeline probiotic industry

SHINKAI, 2013

Study design	Randomised, double blind, placebo controlled, single centre, three-arm parallel trial
Country	Japan
Randomisation	Not clearly described
Blinding	Authors described that Investigators and participants remained blinded to treatment assignment until the end of the study, but they did not provide details on masking.
Duration	March to July 2010
Exclusions post-randomisation	6
Losses to follow-up	14
Participants	Adults aged 65 years or older (mean age 70.8) who resided in Tokyo or its suburbs recruited via a local advertisement. Total number of participants: 300 (High dose intervention=100; Low dose=100; Control=100); none imbalances declared
Intervention	The intervention tablet contained heat-killed *Lactobacillus pentosus* strain b240 tablet with 2 x 10^{10} cells. Participants were instructed to consume one tablet per day at breakfast for 20 weeks.
Infections investigated	Common cold
Outcomes reported	1) Occurrence of common cold; 2) Quality of life; 3) Adverse effects; 4) Medication used during intervention period; 5) Duration; 6) Hospital visits
Power and assumed	Assumed a power of 80%, an average of two common colds per year

risk estimate	on placebo group, and a 25% reduction in the incidence
Other relevant information	
Protocol	Not available
Funders/Support	No specific grant nor funding declared.

THOMAS, 2001

Study design	Randomised, double blind, placebo controlled, single centre, two-arm parallel trial
Country	United States
Randomisation	Randomisation schedule was generated by the Section of Biostatistics abd stratified on three parameters, including baseline daily bowel movement frequency, use of beta-lactams antibiotics and age at entry.
Blinding	A pharmacist who at no time had direct contact with the patients or investigators dispensed active and placebo capsules according to randomisation schedule. Patients and investigators were blinded.
Duration	July 1998 to October 1999
Exclusions post-randomisation	35
Losses to follow-up	35
Participants	Adults (aged 18 to 93 years, mean age 57.2) admitted to a general internal medicine inpatient service, who received an intravenous or oral antibacterial agent for a presumed or proved infection. Total number of participants: 267 (Intervention=152; Control=150); none imbalances declared
Intervention	The intervention capsules contained *Lactobacillus GG*; participants were instructed to consume 1 capsule twice daily for 14 days ($10x10^9$ CFU)
Infections investigated	*Clostridium difficile* infection
Outcomes reported	1) Occurrence of C difficile infection; 2) Duration of hospitalization; 3) Adverse events;
Power and assumed risk estimate	80% power to detect a difference of 20% vs 10% in the rate of occurrence of diahhrea between groups
Other relevant information	Patients included were older than 18 years old. Although aged participants were included, mean age in both groups were younger than 65 years old; the authors did not mention if there were differences according to age (groups were stratified as younger/older than 65 years at enrollment).

Protocol	Not available
Funders/Support	Supported by a grant from CoAgra Foods Inc

VAN PUYENBROECK, 2012

Study design	Randomised, double blind, placebo controlled, multicentre, two-arm parallel trial
Country	Belgium
Randomisation	Random assignment was performed by a third party before enrollment of the volunteers into study.
Blinding	Participants, nursing staff and investigators were blinded to allocation, and the intervention products were identical.
Duration	Winter 2007-2008
Exclusions post-randomisation	0
Losses to follow-up	183 (Intervention=93; Control=90)
Participants	Healthy men and women aged older than 65 years (mean age 83.95) who were living in nursing homes and were willing and able to swallow the study drink twice a day during the study period. Total number of participants: 554 (Intervention=375; Control=362); none imbalances declared
Intervention	The intervention drinks contained *Lactobacillus Casei* strain Shirota $6.5x10^9$ CFU; participants were instructed to consume bottles of 65ml, twice daily, throughout the entire study period.
Infections investigated	Respiratory tract infections
Outcomes reported	1) Probability of acquiring RTI symptoms; 2) Duration of respiratory symptoms
Power and assumed risk estimate	Influenza vaccination could reduce RTI symptoms by 30-60%; power of 80% to detect a reduction higher than 10% on incidence of RTI
Other relevant information	Duration of respiratory symptoms was reported as the number of days with respiratory symptoms/effective number of days of participation multiplied by 100; the occurrence of infection was reported as participants with at least one day of RTI symptoms.
Protocol	NCT00849277
Funders/Support	Supported by Yakult Honsha Co Ltd

Supplementary file 4 - Risk of bias criteria

Adapted from:
Hao Q, Dong BR, Wu T.
Probiotics for preventing acute upper respiratory tract infections.
Cochrane Database of Systematic Reviews 2015, Issue 2. Art. No.: CD006895.
DOI: 10.1002/14651858.CD006895.pub3.

RANDOM SEQUENCE GENERATION
• Low risk of bias: adequate generation of allocation sequence (for example, computer-generated random numbers, table of random numbers, or similar).
• High risk of bias: inadequate generation of allocation sequence (case record number, date of birth, day, month or year of admission, or allocation by judgment of the clinician, the participant, laboratory test or a series of tests, availability of the intervention).
• Unclear risk of bias: the generation of the allocation sequence was unclear.

ALLOCATION CONCEALMENT
• Low risk of bias: adequate concealment of allocation (for example, central independent unit, non-translucent sealed envelopes, or similar).
• High risk of bias: inadequate concealment of allocation (any procedure that is transparent before allocation (for example, alternation, the use of case record numbers, dates of birth, or open table of random numbers or similar).
• Unclear risk of bias: unclear concealment of allocation (for example, only specifying that non-translucent sealed envelopes were used or not reporting any concealment approach) or inadequate.

BLINDING OF PARTICIPANTS AND PERSONNEL
• Low risk of bias: we considered masking of both the participants and study personnel who implemented the study a low risk of performance bias (for example, identical placebo tablets or similar and the study personnel did not know the groups).
• High risk of bias: open-label study.
• Unclear risk of bias: insufficient information provided to judge the level of bias.

BLINDING OF OUTCOME ASSESSMENT
• Low risk of bias: we considered masking of the results assessor a low risk of detection bias.
• High risk of bias: not used or non-blinding of detection of outcomes (for example, not performed or tablets versus fluids or similar).
• Unclear risk of bias: insufficient information provided to judge the level of bias.

INCOMPLETE OUTCOME DATA: ASSESSMENT FOR POTENTIAL BIAS OF EXCLUSION AND ATTRITION
• Low risk of bias: trials had no missing outcome data or few exclusions, attrition is noted and an ITT analysis is possible.
• High risk of bias: there are wide differences in exclusions between the intervention group and control group or the rate of exclusion and/or attrition is higher than 15%, whatever ITT analysis is used.
• Unclear risk of bias: the rate of exclusions or attrition, or both, is higher than 10%, whatever ITT analysis is used.

SELECTIVE REPORTING

• If the protocol for an included study was available, we compared the outcomes in the protocol and published report.

OTHER BIAS

• Any other potential biases, including funding or financial support provided by industry or employment relations between industry and researchers.

Supplementary file 5 - Risk of bias graphs

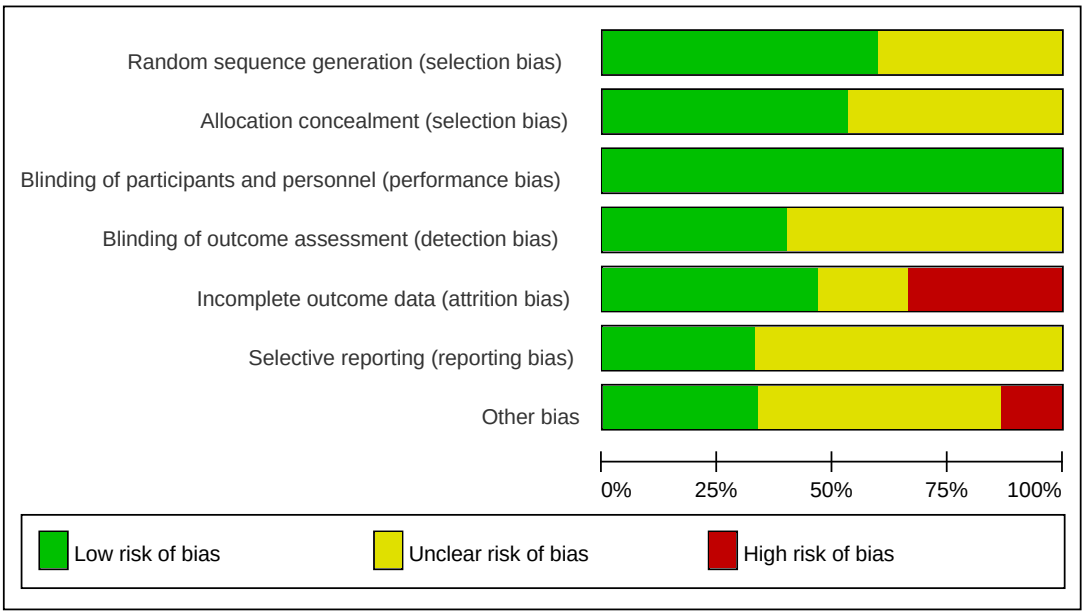

Figure 1. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

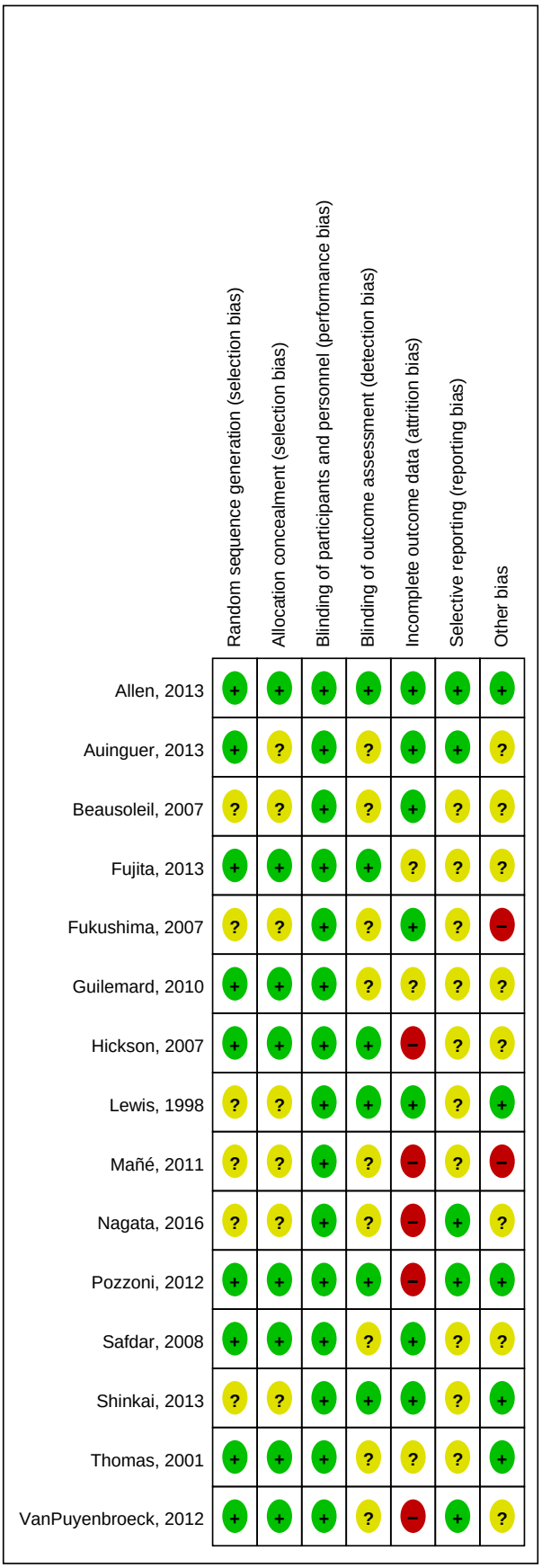

Figure 2. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

Supplementary file 6 - Additional analyses and graphs

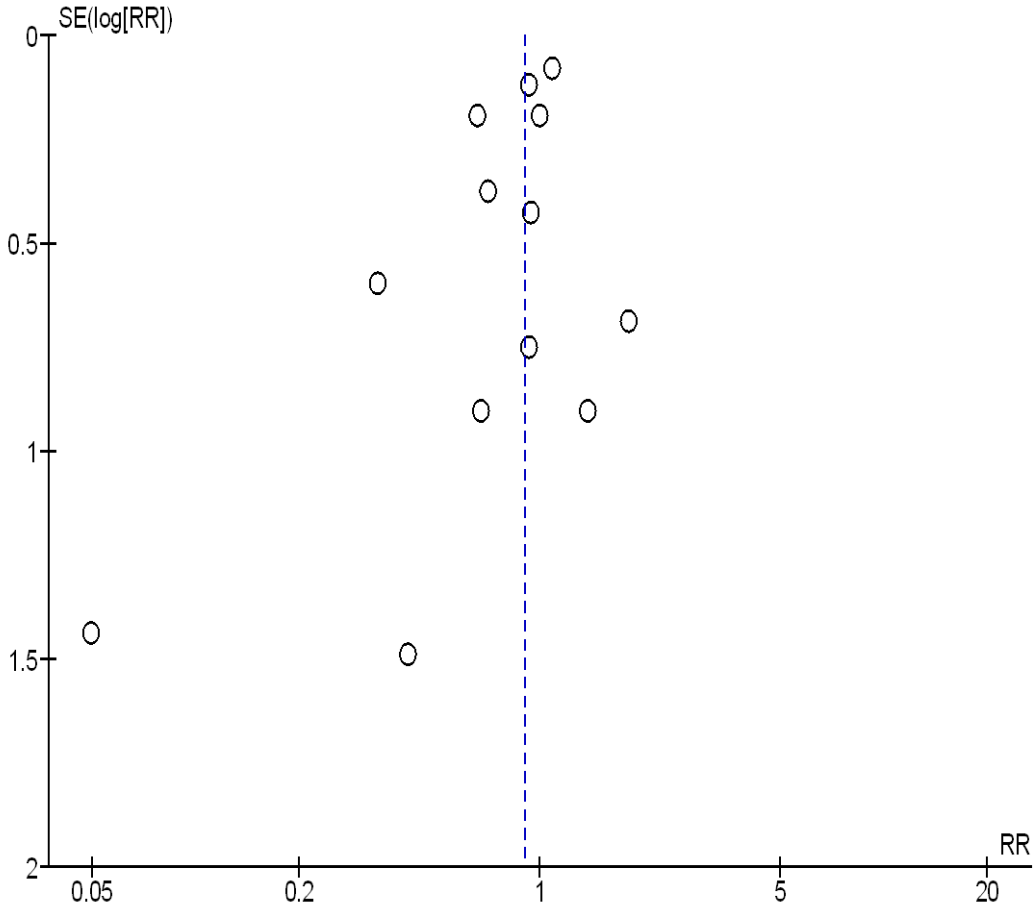

Figure 1. Funnel plot of comparison: Outcome: Prevention of infection. (13 studies)

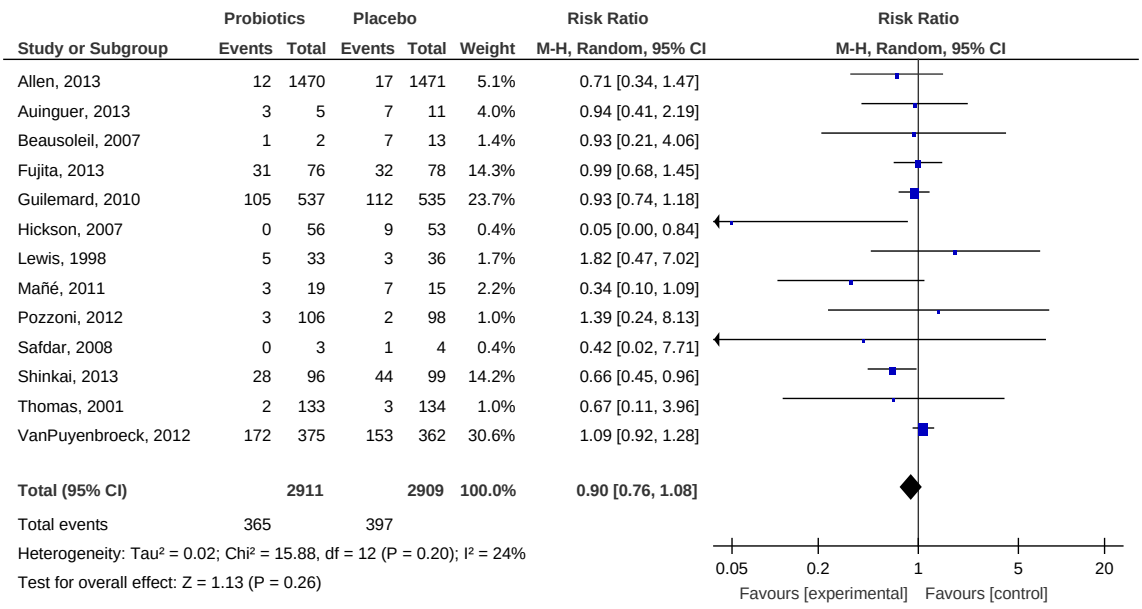

Figure 2. Forest plot of comparison: Outcome: Prevention of infection

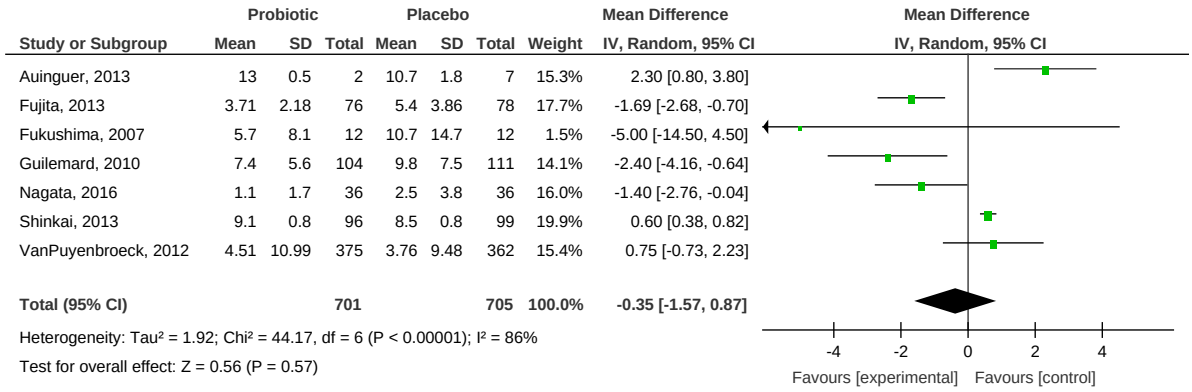

Figure 3. Forest plot of comparison: Outcome: Mean duration of infection per episode

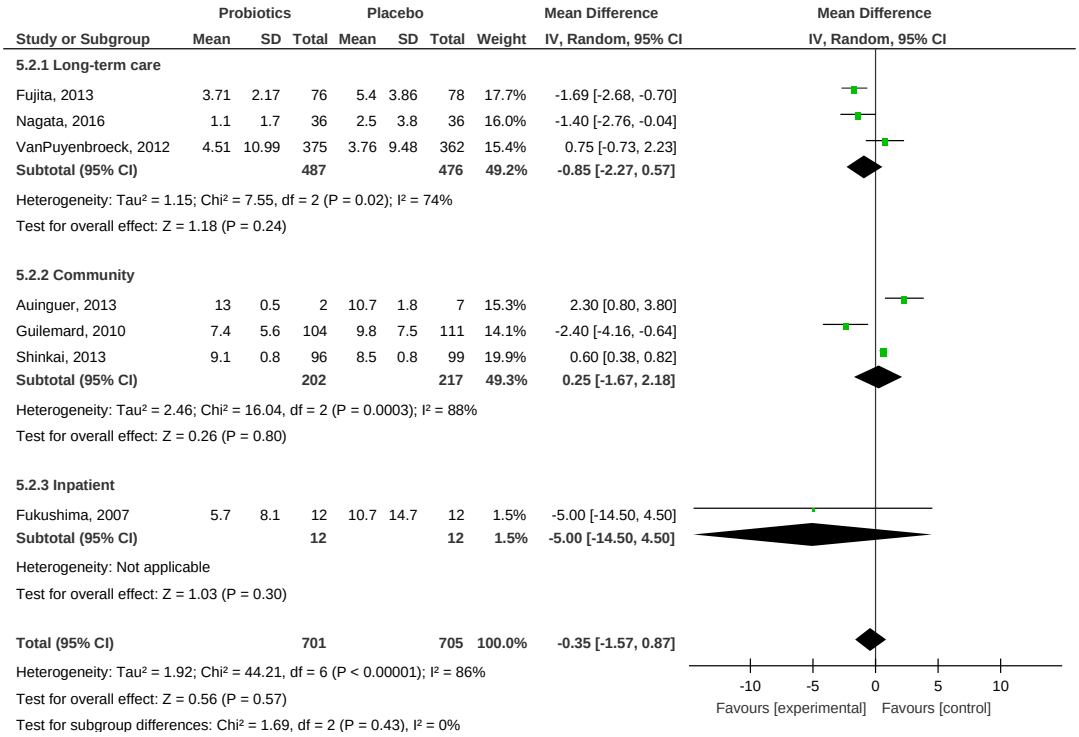

Figure 4.1 Forest plot of comparison: Outcome: Mean duration of infection per episode with subgroup analysis by setting.

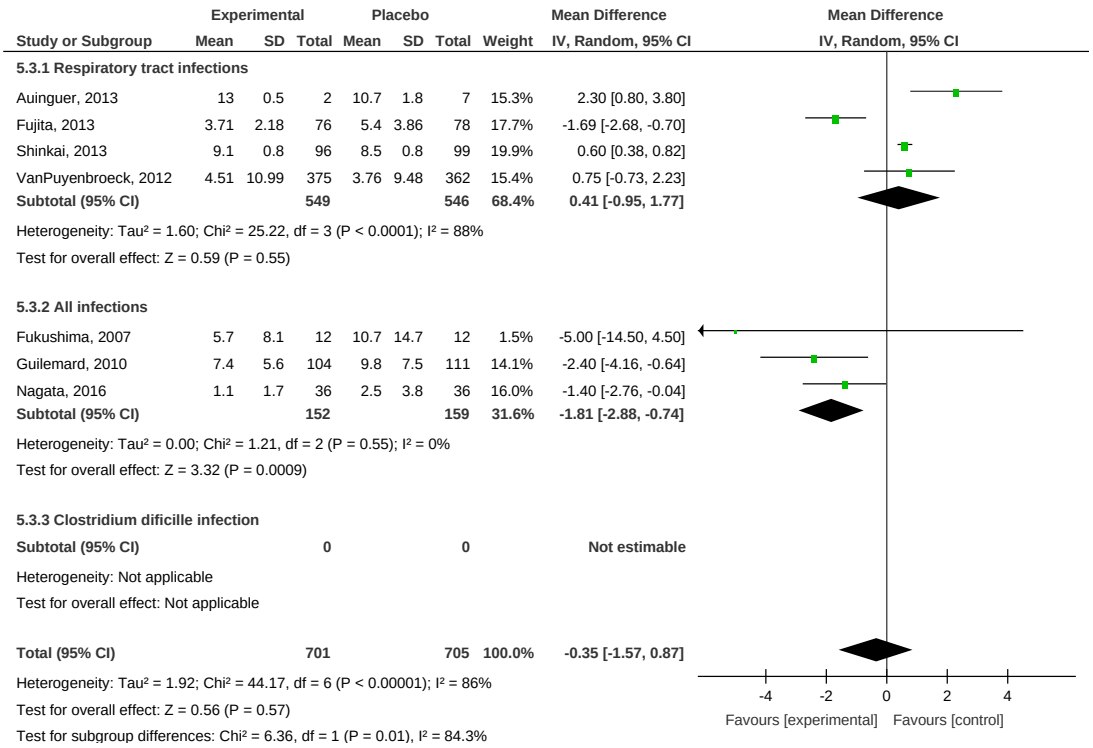

Figure 4.2 Forest plot of comparison: Outcome: Mean duration of infection per episode with subgroup analysis by type of infection.

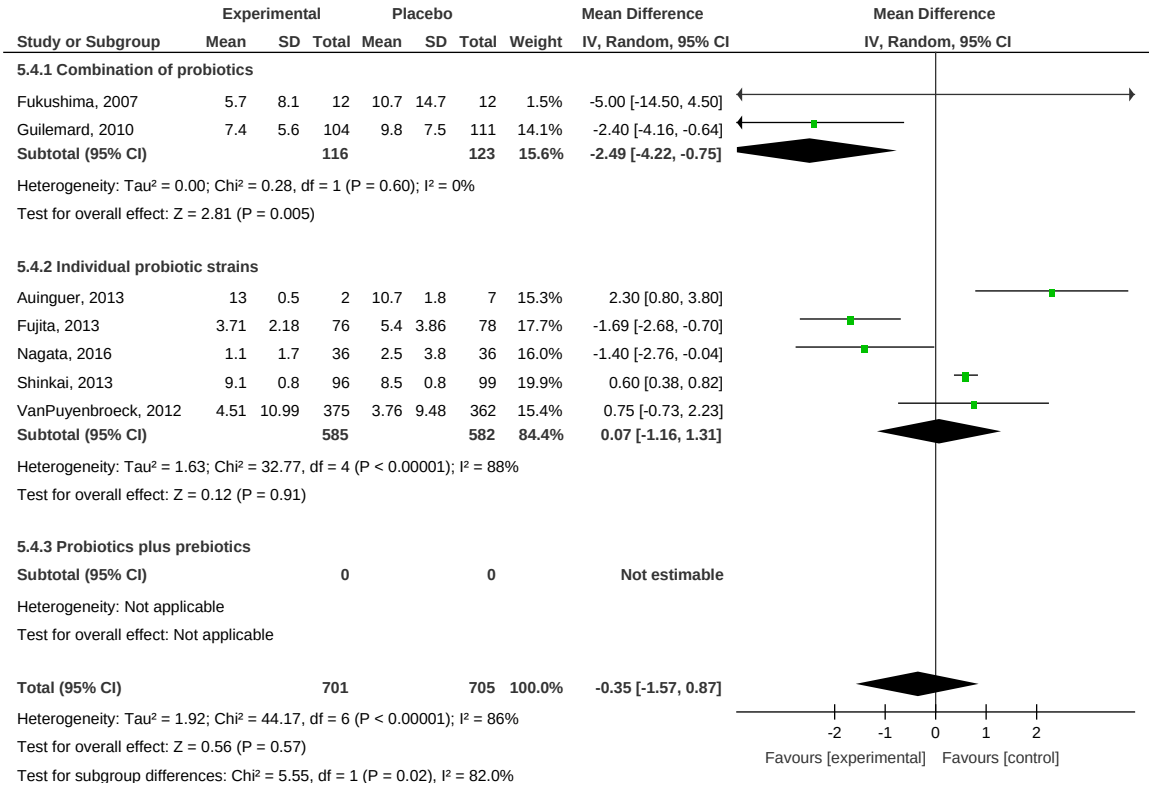

Figure 4.3 Forest plot of comparison: Outcome: Mean duration of infection per episode with subgroup analysis by type of probiotic intervention.

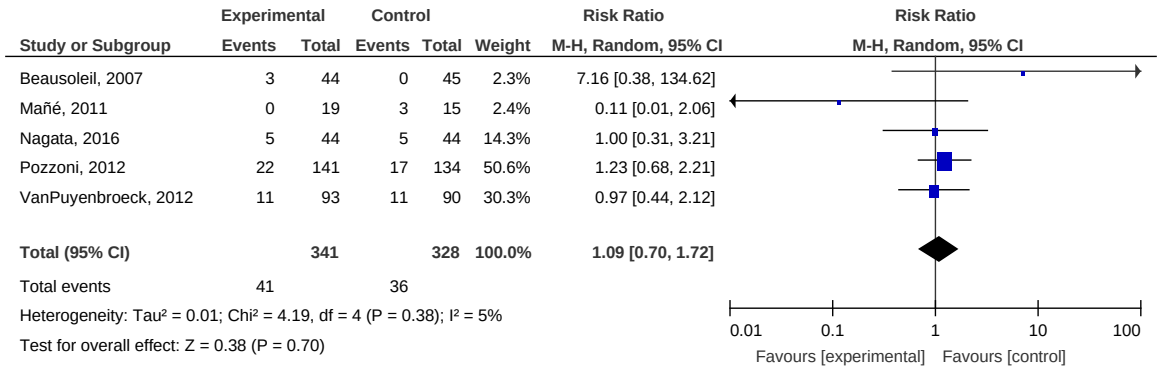

Figure 5. Forest plot of comparison: Outcome: Mortality

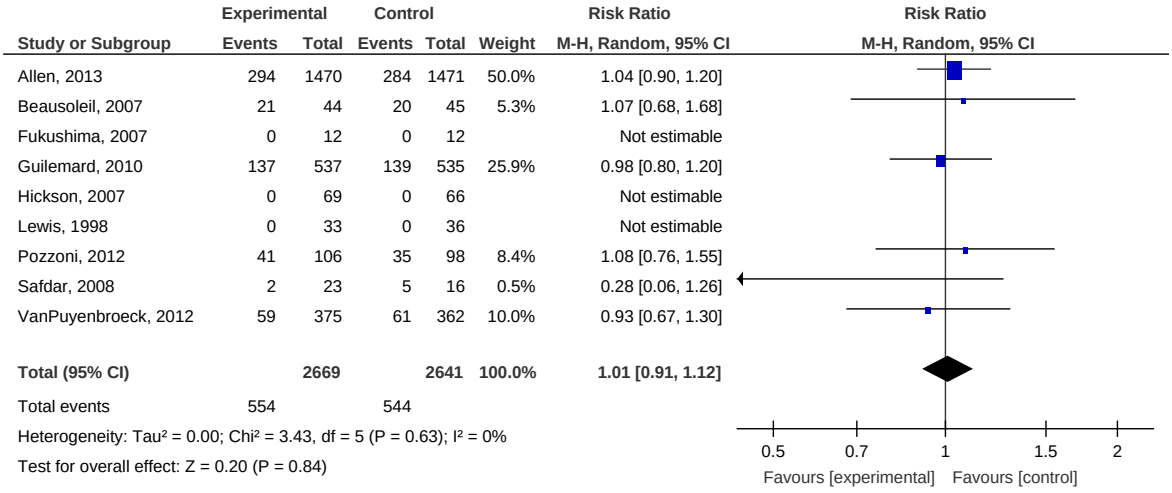

Figure 6. Forest plot of comparison: Outcome: Adverse events

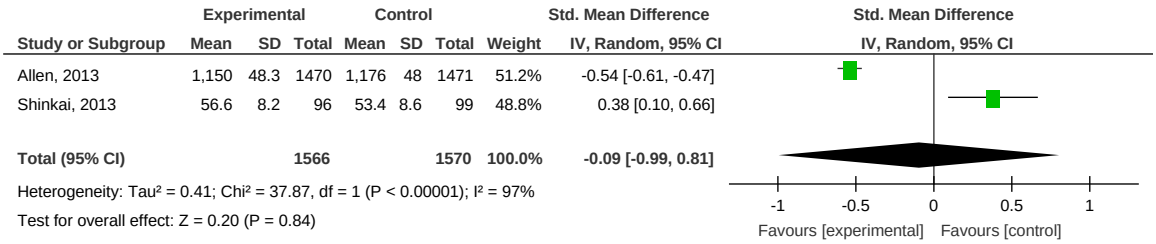

Figure 7. Forest plot of comparison: Outcome: Quality of life

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Apêndices

Apêndice 1

Estratégia de busca - MEDLINE (via PUBMED)

# 1	**"Aged"[Mesh] OR "Aged, 80 and over"[Mesh] OR (older) OR (senior*) OR (elderly) OR (geriatric) OR (Centenarians) OR (Nonagenarians) OR (Octogenarians) OR (Oldest Old)**
# 2	**"Probiotics/administration and dosage"[Mesh] OR "Probiotics/therapeutic use"[Mesh] OR "lactobacillus"[Mesh] OR "lactococcus"[Mesh] OR "bifidobacterium"[Mesh] OR "enterococcus"[Mesh] OR "streptococcus"[Mesh] OR "saccharomyces"[Mesh] OR (lactobacill*) OR (lactococc*) OR (bifidobacter*) OR (enterococc*) OR (streptococc*) OR (saccharomyces)**
# 3	**"Infection/prevention and control"[Mesh] OR (infection prevention) OR (infection control)**
# 4	**randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [pt] OR evaluation studies as topic [mh] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])**
	(((((("Aged"[Mesh] OR "Aged, 80 and over"[Mesh] OR (older) OR (senior*) OR (elderly) OR (geriatric) OR (Centenarians) OR (Nonagenarians) OR (Octogenarians) OR (Oldest Old)))) AND

	(("Probiotics/administration and dosage"[Mesh] OR "Probiotics/therapeutic use"[Mesh] OR "lactobacillus"[Mesh] OR "lactococcus"[Mesh] OR "bifidobacterium"[Mesh] OR "enterococcus"[Mesh] OR "streptococcus"[Mesh] OR "saccharomyces"[Mesh] OR (lactobacill*) OR (lactococc*) OR (bifidobacter*) OR (enterococc*) OR (streptococc*) OR (saccharomyces)))) AND (("Infection/prevention and control"[Mesh] OR (infection prevention) OR (infection control)))) AND (((randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR (placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [pt] OR evaluation studies as topic [mh] OR follow-up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw]) NOT (animals [mh] NOT humans [mh])))

Apêndice 2

Estratégia de busca - EMBASE

# 1	**('elderly'**/exp) OR (**'very elderly'**/exp) OR **('centenarians'**/exp) OR **('nonagenarians'**/exp) OR **('octogenarians'**/exp) OR **('older')** OR (**'senior')** OR **('elderly')** OR (**'geriatric')** OR (**'centenarians')** OR **('nonagenarians')** OR (**'octogenarians')** OR (**'oldest old')** OR (**'very elderly')**
# 2	**('probiotic agent'**/exp) OR (**'probiotic agent')** OR (**'lactobacillus'**/exp) OR (**'lactobacillus')** OR (**'lactococcus'**/exp) OR (**'lactococcus')** OR (**'bifidobacterium'**/exp) OR (**'bifidobacterium')** OR (**'enterococcus'**/exp) OR (**'enterococcus')** OR (**'streptococcus'**/exp) OR (**'streptococcus')** OR (**lactococc*)** OR (**bifidobacter*)** OR (**enterococc*)** OR (**streptococc*)** OR (**'saccharomyces'**/exp) OR (**'saccharomyces')**
# 3	**('infection prevention'**/exp) OR (**'infection prevention')** OR (**'infection control'**/exp) OR (**'infection control')**
	[embase]/lim NOT [medline]/lim
	#1 AND #2 AND #3

Apêndice 3

Estratégia de busca - LILACS

	MH:"aged" OR MH: "aged,80 and over" OR (older) OR (senior) OR (elderly) OR (centenarian*) OR (nonagenarian*) OR (octogenarian*) OR (oldest old) OR MH:M01.060.116.100.080 OR MH:01.060.116.100
	MH:"Probiotics/administration & dosage" OR MH:"Probiotics/therapeutic use" OR (probiotic$) OR MH:"lactobacillus" OR MH:"bifidobacterium" OR MH:"enterococcus" OR MH:"streptococcus" OR MH:"saccharomyces" OR tw:(lactobacil*) OR tw:(lactococc*) OR tw:(bifidobacter*) OR tw:(enterococc*) OR tw:(streptococc*) OR tw:(saccharomyces) OR MH:J02.500.456.500 ORMH:B03.510.460.400.410.475.475 OR MH:B03.510.550.450.475 OR MH: B03.510.400.800.500 OR MH:B03.510.550.737.500 OR MH:B03.510.024.100 OR MH:B03.510.460.400.400.049.100 OR MH:B03.510.550.250.250 OR MH:B03.510.400.800.872 OR MH:B03.510.550.737.872 OR MH:B01.300.107.795.785 OR MH:B01.300.930.705
	MH:"Infection control" OR MH:"infection" OR (infection prevention) OR (infection) OR MH:N06.850.780.200.450 OR MH:C01.539

Apêndice 4

Relação dos estudos excluídos e razões para exclusão

Estudo	Razões para exclusão
Allen, 2013[1]	Dados duplicados
Allen, 2013[2]	Dados duplicados
Anderson, 2004[3]	Critérios de exclusão: pacientes cirúrgicos
Askerov, 2011[4]	Autores não responderam a três tentativas distintas de contato
Barraud, 2010[5]	Critério de exclusão: pacientes criticamente enfermos
Bleichner, 1997[6]	Critério de exclusão: pacientes criticamente enfermos
d´Ettore, 2015[7]	Critérios de Exclusão: pacientes imunossuprimidos
Ehrhardt, 2016[8]	Idade dos participantes não relevante
Flatley, 2015[9]	Delineamento do estudo não relevante
Forestier, 2008[10]	Critério de exclusão: pacientes criticamente enfermos
Gerritsen, 2016[11]	Delineamento do estudo não relevante
Giamarellos, 2009[12]	Critério de exclusão: pacientes criticamente enfermos
Graubaum, 2012[13]	Idade dos participantes não relevante
Hilton, 1992[14]	Idade dos participantes não relevante
Ishikawa, 2015[15]	Desfechos não relevantes
Jain, 2004[16]	Critério de exclusão: pacientes criticamente enfermos
Kanazawa, 2005[17]	Critérios de exclusão: pacientes cirúrgicos
Kenna, 2016[18]	Critério de exclusão: pacientes criticamente enfermos
Kwon, 2015[19]	Critério de exclusão: pacientes criticamente enfermos
Knight, 2009[20]	Critério de exclusão: pacientes criticamente enfermos
Kotzampassi, 2015[21]	Critérios de exclusão: pacientes cirúrgicos
Liu, 2011[22]	Critérios de exclusão: pacientes cirúrgicos
Liu, 2013[23]	Critérios de exclusão: pacientes cirúrgicos
Liu, 2015[24]	Critérios de exclusão: pacientes cirúrgicos
Makino, 2010[25]	Delineamento do estudo não relevante
Mego, 2015[26]	Critérios de exclusão: pacientes cirúrgicos
Merenstein, 2015[27]	Desfechos não relevantes
Morita, 2016[28]	Idade dos participantes não relevante; comparador não válido
Morrow, 2010[29]	Critério de exclusão: pacientes criticamente enfermos
Murina, 2014[30]	Idade dos participantes não relevante
Nomura, 2007[31]	Critérios de exclusão: pacientes cirúrgicos
Okazaki, 2013[32]	Critérios de exclusão: pacientes cirúrgicos
Oláh, 2002[33]	Critérios de exclusão: pacientes cirúrgicos
Oláh, 2005[34]	Critérios de exclusão: pacientes cirúrgicos
Oláh, 2007[35]	Critérios de exclusão: pacientes cirúrgicos
Przemska-Kosicka, 2016[36]	Desfechos não relevantes
Rayes, 2002[37]	Critérios de exclusão: pacientes cirúrgicos
Rayes, 2005[38]	Critérios de exclusão: pacientes cirúrgicos
Rayes, 2007[39]	Critérios de exclusão: pacientes cirúrgicos
Rongrungruang, 2015[40]	Critério de exclusão: pacientes criticamente enfermos
Roos, 2011[41]	Desfechos não relevantes
Salomão, 2016[42]	Critério de exclusão: diagnóstico de infecção na admissão
Selinger, 2013[43]	Idade dos participantes não relevante
Smith, 2016[44]	Desfechos não relevantes
Sommacal, 2015[45]	Critérios de exclusão: pacientes cirúrgicos
Sugawara, 2006[46]	Critérios de exclusão: pacientes cirúrgicos

Sullivan, 2002[47]	Desfechos não relevantes
Tanaka, 2012[48]	Critério de exclusão: pacientes cirúrgicos
Tubelius, 2005[49]	Idade dos participantes não relevante
Turchet, 2003[50]	Comparador não válido
Valdes-Varella, 2016[51]	Delineamento não relevante
Wang, 2015[52]	Desfechos não relevantes
Wright, 2015[53]	Desfechos não relevantes
Yang, 2016[54]	Critério de exclusão: pacientes cirúrgicos
Zhang, 2010[55]	Critério de exclusão: pacientes cirúrgicos
Zeng, 2016[56]	Critério de exclusão: pacientes criticamente enfermos

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Apêndice 5

Resultados da aplicação do instrumento de avaliação da qualidade metodológica "*Effective Public Health Practice Project Quality Assessment tool* (EPHPP-QAt)"

Título	Referência Completa	**EPHPP Global Rating** **1 FORTE** **2 MODERADA** **3 FRACA**
Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial	Allen SJ, Wareham K, Wang D, Bradley C, Hutchings H, Harris W, et al. Lactobacilli and bifidobacteria in the prevention f antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2013 Oct 12;382(9900):1249–57.	2
Decreased duration of acute upper respiratory tract infections with daily intake of fermented milk: A multicenter, double-blinded, randomized comparative study in users of day care facilities for the elderly population	Fujita R, Iimuro S, Shinozaki T, Sakamaki K, Uemura Y, Takeuchi A, et al. Decreased duration of acute upper respiratory tract infections with daily intake of fermented milk: A multicenter, double-blinded, randomized comparative study in users of day care facilities for the elderly population. Am J Infect Control. 2013 Dec;41(12):1231–5.	1
Improvement of nutritional status and incidence of infection in hospitalised, enterally fed elderly by feeding of fermented milk containing probiotic Lactobacillus johnsonii La1 (NCC533)	Fukushima Y, Miyaguchi S, Yamano T, Kaburagi T, Iino H, Ushida K, et al. Improvement of nutritional status and incidence of infection in hospitalised, enterally fed elderly by feeding of fermented milk containing probiotic Lactobacillus johnsonii La1 (NCC533). Br J Nutr. 2007 Nov;98(5):969–77.	2
Consumption of a fermented dairy product containing the probiotic Lactobacillus casei DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial	Guillemard E, Tondu F, Lacoin F, Schrezenmeir J. Consumption of a fermented dairy product containing the probiotic Lactobacillus casei DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial. Br J Nutr. 2010 Jan;103(1):58–68.	1
Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial	Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, et al. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ. 2007 Jul 14;335(7610):80.	2
The lack of therapeutic effect of Saccharomyces boulardii in the prevention of antibiotic-related diarrhoea in elderly patients	Lewis S, Potts L, Barry R. The lack of therapeutic effect of Saccharomyces boulardii in the prevention of antibiotic-related diarrhoea in elderly patients. J Infect. 1998 Mar;36(2):171–4.	3

A mixture of Lactobacillus plantarum CECT 7315 and CECT 7316 enhances systemic immunity in elderly subjects. A dose-response,double-blind, placebo-controlled, randomized pilot trial	Mañé J, Pedrosa E, Lorén V, Gassull MA, Espadaler J, Cuñé J, et al. A mixture of Lactobacillus plantarum CECT 7315 and CECT 7316 enhances systemic immunity in elderly subjects. A dose-response, double-blind, placebo-controlled, randomized pilot trial. Nutr Hosp Organo Of Soc Esp Nutr Parenter Enter. 2011 Feb;26(1):228–35	3
Saccharomyces boulardii for the Prevention of Antibiotic-Associated Diarrhea in Adult Hospitalized Patients: A Single-Center, Randomized, Double-Blind, Placebo-Controlled Trial	Pozzoni P, Riva A, Bellatorre AG, Amigoni M, Redaelli E, Ronchetti A, et al. Saccharomyces boulardii for the Prevention of Antibiotic-Associated Diarrhea in Adult Hospitalized Patients: A Single-Center, Randomized, Double-Blind, Placebo-Controlled Trial. J Gastroenterol. 2012;107(6):922–31.	2
Feasibility and tolerability of probiotics for prevention of antibiotic-associated diarrhoea in hospitalized US military veterans	Safdar N, Barigala R, Said A, McKinley L. Feasibility and tolerability of probiotics for prevention of antibiotic-associated diarrhoea in hospitalized US military veterans. J Clin Pharm Ther. 2008 Dec;33(6):663–8.	3
Immunoprotective effects of oral intake of heat-killed Lactobacillus pentosus strain b240 in elderly adults: A randomised, double-blind, placebo-controlled trial	Shinkai S, Toba M, Saito T, Sato I, Tsubouchi M, Taira K, et al. Immunoprotective effects of oral intake of heat-killed Lactobacillus pentosus strain b240 in elderly adults: a randomised, double-blind, placebo-controlled trial. Br J Nutr. 2013 May 28;109(10):1856–65.	1
Lack of effect of Lactobacillus GG on antibiotic-associated diarrhea: a randomized, placebo-controlled trial	Thomas, M. R., Litin, S. C., Osmon, D. R., Corr, A. P., Weaver, A. L. and Lohse, C. M. Mayo Clinic proceedings.2001; 76: 883-9	3
Efficacy of daily intake of Lactobacillus casei Shirota on respiratory symptoms and influenza vaccination immune response: a randomized, double-blind, placebo-controlled trial in healthy elderly nursing home residents	Van Puyenbroeck K, Hens N, Coenen S, Michiels B, Beunckens C, Molenberghs G, et al. Efficacy of daily intake of Lactobacillus casei Shirota on respiratory symptoms and influenza vaccination immune response: a randomized, double-blind, placebo-controlled trial in healthy elderly nursing home residents. Am J Clin Nutr. 2012 May;95(5):1165–71.	1
Yeast (1,3)-(1,6)-beta-glucan helps to maintain the body's defence against pathogens: a double-blind, randomized, placebo-controlled, multicentric study in healthy subjects	Auinger A, Riede L, Bothe G, Busch R, Gruenwald J. Yeast (1,3)-(1,6)-beta-glucan helps to maintain the body’s defence against pathogens: a double-blind, randomized, placebo-controlled, multicentric study in healthy subjects. Eur J Nutr. 2013 Dec;52(8):1913–8.	3

Effect of a fermented milk combining Lactobacillus acidophilus CL1285 and Lactobacillus casei in the prevention of antibiotic-associated diarrhea: a randomized, double blind, placebo-controlled trial [BUSCA MANUAL]	Beausoleil M, Fortier N, Guénette S, L´Ecuyer A, Savoie M, Franco M, Lachaine J, Weiss K. Effect of a fermented milk combining Lactobacillus acidophilus CL1285 and Lactobacillus casei in the prevention of antibiotic-associated diarrhea: a randomized, double blind, placebo-controlled trial. Can J Gastroenterol. 2007; 21(11): 732-736	2
The effectiveness of Lactobacillus beverages in controlling infections among the residents of na aged care facility: a randomized placebo-controlled double-blind trial	Nagata S, Asahara T, Wang C, Suyama Y, Chonan O, Takano K, Daibou M, Takahashi T, Nomoto K, Yamashiro Y. The effectiveness of Lactobacillus beverages in controlling infections among the residents of na aged care facility: a randomized placebo-controlled double-blind trial. Ann Nutr Metab. 2016; 68:51-59.	3

Anexos

Anexo 1

Clinical Nutrition 32 (2013) 1082

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Letter to the Editor

Effects of symbiotic food consumption in diabetic patients

To the Editor,

We read with interest the article by Asemi et al.[1] who reported that consumption of a symbiotic food among diabetic patients had significant effects on serum insulin, high sensitivity C-reactive protein, uric acid and Glutathione levels. Although we appreciate their work, there are a few limitations that need comments.

First, the evaluation of oxidative stress performed by the authors was based only in total antioxidant capacity and total glutathione levels. Oxidative stress can be assessed using a wide variety of methods, such as isoprostanes, malondialdehyde, ferric reducing antioxidant power, oxygen radical absorbance capacity, antioxidant enzymes: catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, antioxidant vitamins and the minerals selenium and zinc. Oxidative stress can be assessed using a wide variety of methods, such as isoprostanes, malondialdehyde, ferric reducing antioxidant power, oxygen radical absorbance capacity, antioxidant enzymes: catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, antioxidant vitamins and the minerals selenium and zinc.[2] Current standards for the assessment of oxidative stress recommend that conclusions regarding the effects of medical interventions on oxidative stress be based on multiple biomarkers.[3]

Second, the authors might have neglected the impact of medications in the results of this study. Despite the fact that patients using insulin were excluded, some anti-hyperglycemic medications, statins and aspirin are known to influence metabolic profile, inflammatory and oxidative stress markers.[4]

Third, there seems to be a decline in gut microbiota diversity with aging that may affect the ability of individuals to synthesize vitamins and anti-oxidant products. Therefore symbiotic foods may exert different effects in younger and older adults.[5] It would have been very interesting if the authors had provided a sensitivity analysis comparing the outcomes between younger and older people.

Finally, what concerns us most is the fact that the authors did not assess the possibility of a carryover effect between the cross-over phases of the study. This is an important limitation of that study, which can significantly compromise its results. The washout period of 3 weeks chosen by the authors might simply not have been enough to avoid that any effect of the intervention during the first phase of the study was carried over into the second phase. This is a classic problem of cross-over clinical trials and probably the main reason why they are not more commonly performed.

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Anexo 2

Journal of Clinical Gerontology & Geriatrics 5 (2014) 1–2

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Letter to the Editor

Evidence on the role of prebiotics, probiotics, and synbiotics in gut health and disease prevention in the elderly

To the Editor

In their review article, Patel et al[1] elegantly described the process of ageing of the small bowel under normal conditions. In order to properly introduce the topic, it is reasonable to add information about the aging stomach and colon, as evidence shows age-related declines in these structures.

Along with hypochlorhydria (that can predispose the elderly to small intestine overgrowth, Fe malabsorption, and vitamin B12 deficiency), evidence suggests that reduction in gastric compliance, particularly of the fundus, plays an important role in the anorexia of ageing and satiety response.[2] The loss of neurons in both submucosal, gastric and enteric plexus begins early in life, along with a reduction in the expression of acetylcholine (ACh) and nitric oxide (NO).[1,2] Furthermore, orexigenic factors like neuropeptide Y decline with ageing, and leptin (a hormone related to satiety) have been shown to increase, particularly in men, probably due to a decrease in testosterone.[2]

The increase in colonic transit time and decline in propulsive activity are hypothesized to be related to neurodegeneration and reduction in NO and ACh, making the elderly prone to constipation.[2] The colon plays little role in nutrition, but is an important element for gut microbiome; the number of colony-forming units (CFU) increases from 10^2 CFU in the upper small bowel to 10^{12} CFU in the colon.[2]

As the authors stated,[1] the gut microbiota plays an important role in the maintenance of the host health and disease in all age groups, but particularly in the elderly.[3] The mechanisms related to the benefits of probiotics are incompletely understood. However, four general benefits have been described: (1) suppression of growth or epithelial binding/invasion by pathogenic bacteria, (2) improvement of intestinal barrier function, (3) modulation of the immune system, and (4) modulation of pain perception.[1,3,4]

The majority of bacteria in the colon are anaerobes that can ferment carbohydrates that escape digestion, to form short chain fatty acids (SCFAs) and anions that have a distinct role in promoting gut health (e.g., acetate, propionate, and butyrate).[3] Studies in the elderly described a shift in the composition of intestinal microbiota, with a lower number of beneficial organisms such as bifidobacteria and lactobacilli[1] and an increase in Enterobacteriaceae and certain Proteobacteria.[3] Compared to younger adult controls, aged persons seem to have a lower number of Firmicutes and more abundant Bacteroidetes.[3] Butyrate is the major energy source for the colonic epithelium, and its levels are usually lower in the elderly, with concomitant increased levels of SCFAs, ammonia, and phenols.[3] Evidence showed that lactate accumulation in the colon might be indicative of gut microbiome imbalance, and it was suggested that this could be a target for the development of novel probiotics to prevent lactate accumulation.[3]

Despite previous good to moderate evidence of the beneficial effects of probiotic and synbiotic administration, recent larger randomized controlled trials reported contradictory results related to the reduction in the risk of infections [*Clostridium difficile*-associated diarrhea (CDAD), upper respiratory tract infections, antibiotic-associated-diarrhea (AAD), etc.]. The *PLACIDE Trial* reported no evidence of a multi strain preparation of lactobacilli and bifidobacteria in the prevention of AAD and CDAD.[4] Another recent large trial evaluated the evidence of the preventive effect of probiotics on upper respiratory tract infections (URTI) in an aged sample, and was unable to find differences between groups.[5] Only in the subgroup analysis did the authors find that probiotics probably reduced the duration of acute URTI.[5]

Despite good evidence of the clinical benefit of probiotic use in children and under some specific conditions, there are no meta-analysis and systematic reviews, to date, that address the use of probiotics in the elderly for infection prevention and control. This issue, we hope, will soon be covered by a systematic review protocol that we are already developing.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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Anexo 3

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

PROSPERO International prospective register of systematic reviews

Effectiveness of probiotics to prevent infections in the elderly: systematic review and meta-analysis

Patrick Alexander Wachholz, Paulo José Fortes Villas Boas, Vânia dos Santos Nunes, Hélio Rubens de Carvalho Nunes, Adriana Polachini Valle

Citation

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Review question(s)

Is the use of probiotics effective in preventing infections in the elderly living in the community or in long-term care facilities?

Searches

We will comply with the recommendations of the Cochrane Collaboration, especially with the "Methodological Expectations of Cochrane Intervention Reviews" - MECIR (Chandler 2013), and the principles of the PRISMA Statement (Moher 2009).

Search methods for identification of studies:

We will attempt to identify all relevant studies, regardless of language, publishing period or publication status (published, unpublished, in press, ongoing). The electronic search will be conducted by the main reviewer (PAW), and searches for unpublished trials, reference lists and grey literature will be performed by three authors (PAW, PJFVB, VSN). We will use specially designed and tested search filters (e.g., Cochrane Highly Sensitive Search Strategies for identifying randomized trials in MEDLINE), where appropriate.

Electronic searches:

We will search the following databases for relevant studies: the Cochrane Central Register of Controlled Trials (CENTRAL) published in The Cochrane Library; MEDLINE (PubMed); EMBASE (OVID); ISI Web of Science and LILACS, using the search terms detailed in the accompanying PDF. We will also search the WHO clinical trial registry platform and the metaRegister of Controlled Trials (mRCT) for ongoing trials.

Searching other resources:

We will check the reference lists of all studies identified by the above methods for other potentially relevant studies. In addition, we will search for information about unpublished studies in the World Health Organization International Clinical Trials Registry Platform, Clinical Trials and Current Controlled Trials and Pharmaceutical Abstracts (1970 to present). We will also ask experts and contact pharmaceutical companies for further information on grey literature and unpublished trials in scientific conferences in OpenSigle (1993 to present) and Google Scholar.

Types of study to be included

We will include only randomised controlled trials (RCTs). Non-standard designs, such as cluster RCTs, and cross-over RCT, will not be included due to the risk that the effects of the interventions in these studies could result in unit of analysis error, or inflate standard errors.

Condition or domain being studied

The proportional and absolute number of people aged over 65 years is growing at an unprecedented rate, especially in the subgroup older than 85 years (Liang 2007). The burden of aging found on previous economic evaluations in

public health showed that this age group was responsible for one of every three inpatient stays, accounting for almost $329 billion in hospital charges, suggesting that, by 2020, three-quarters of overall mortality in developing countries may be due to age-associated diseases (Gavazzi 2004; Liang 2007).

Although noncommunicable causes of death (such as cancer, heart failure, and diabetes) are predominant among elders from developed countries, the impact of infections in morbidity and mortality is more significant in the developing world: almost five percent from Europe and United States elderly population will die as consequence to infectious diseases, while this proportion at Africa is expected to be around 20% (WHO 2001; Videcnik Zorman 2013). In other words, infections in the elderly will contribute significantly to the increase in societal and health costs in developing countries in the coming decades (Gavazzi 2004).

An infection could be defined by clinical, laboratory and complementary parameters, depending mainly, but not exclusively, on the immune response, type of infection and site. In older patients, infections may present with atypical manifestations: for example, fever may be absent or blunted in elderly patients, what complicates a prompt and accurate diagnosis of infection in this age group (Yoshikawa 1997).

Infections in the elderly could be assumed or established by symptomatic diagnostics, microbial culture, microscopy, biochemical tests and molecular diagnostics. Definitions used in trials sometimes are not feasible at the clinical setting, but most remain useful: the appearance of new or acute worsening of symptoms and/or signs suggestive of infection (for example, productive cough, rales, diarrhea, redness/swelling/drainage or tenderness, pyuria,etc). In older persons, a decline in functional status [e.g., walking, dressing, bathing, etc], new or worsening confusion, and reduced food intake could also be signs of infections (Videcnik Zorman 2013).

Older persons have greater susceptibility to infections compared to younger adults, and generally more severe and atypical episodes (Yoshikawa 1997; Htwe 2007; Steens 2014). With aging, the ability to ward off infectious agents is compromised by changes in nonadaptative immunity, impact of chronic diseases, effects of medication, malnutrition and functional deficits (Yoshikawa 1997; Strausbaugh 2001; Liang 2007; Steens 2014). In this regard, the immune system functional dysregulation has been assumed as a plausible cause for increased susceptibility of the elderly to infectious diseases (Pawelec 2010; Pae 2012; Dewan 2012).

Despite current use of more effective antimicrobial agents, advances in diagnostic technologies and better preventive measures, infections stand out as one of the major causes of morbidity and mortality in the elderly, particularly in long-term care units (Laurent 2012). Studies on community-acquired infections in the aged suggest that the most common site was respiratory tract (40-43%), followed by urinary tract (22-27%) and skin and soft tissues infections (13-15%) (Liang 2007; Videcnik Zorman 2013). Although the majority of elderly population live in the community, long-term care residents are more often admitted to emergency departments and hospital units due to infections. The profiles of these diseases are different, and they have higher severity and associated-mortality (Yoshikawa 1997; Videcnik Zorman 2013; Steens 2014).

Participants/ population

Types of participants:

We will include studies that recruited elderly people (aged 65 years or more), regardless of gender, comorbidities and functional or cognitive status, who live in the community or are institutionalised, and that have no clinical or laboratory signs and/or diagnosis of infections at baseline. Patients that develop nosocomial infections will be excluded from this review.

In studies conducted with different age groups, only data relevant to the elderly segment will be collected, using suitable statistical procedures for extracting these data, and seeking additional information with the authors, when they are not available in the full text articles.

Intervention(s), exposure(s)

The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations define probiotics as live microorganisms that confer a health benefit to the host when administered in sufficient quantities (WHO 2001).

Probiotics should be able to reach the digestive tract alive, remaining viable during passage through the stomach and exposure to bile (WHO 2001; Bermudez-Brito 2012; Duncan 2013). Evidence suggests that even bacterial components or heat-inactivated bacteria may exert health benefits (Duncan 2013).

Several probiotics are available worldwide, and the most commonly reported strains are: the Lactobacillus species acidophilus, bifidum, brevis, buchneri, bulgaricus, casei subsp. paracasei and subsp. tolerans, caucasicus, coryniformis, crispatus, delbrueckii, fermentum, gasseri, (GG), helveticus, johnsonii, lactis, leichmannii, paracasei, plantarum, reuteri, rhamnosus, and salivarius; the Bifidobacterium species animalis, bifidum, breve, clausii, infantis, lactis, and longum; the Saccharomyces species boulardii, cerevisiae, or cerevisiae boulardii; the Enterococcus species faecalis and faecium.

There are also Streptococcus species: mitis, oralis, rattus, salivarius, sanguis, thermophilus, and faecium, and Bacillus species: clausii, coagulans, licheniformis, oligonitrophilus, stearothermophilus, and subtilis.Typical doses used in studies ranged from least 107- 1010colony-forming units/g (WHO 2001; Bermudez-Brito 2012). Probiotics could be found in commercially available preparations or manipulated products.

The use of probiotics with the intention of obtaining health benefits can be achieved with isolated strains, with the use of multiple strains, and combining probiotics with other substances, such as prebiotics (Bermudez-Brito 2012; Duncan 2013). Prebiotics are non-digestible fiber compounds, that stimulate the growth and/or activity of advantageous bacteria, acting as substrate for them, and increasing the number and/or activity of lactic acid bacteria, like the strains of probiotics bacteria.

Types of interventions to be reviewed:

Any intervention with specific and identified probiotic strains (such as Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Bacillus and/or Lactococcus), at any effective dose regimen (at least 107 colony-forming units/g), using a intervention with single or multiple strains, alone or in combination with prebiotics, regardless of the duration and dosage of treatment (single or multiple doses), by any route of administration (except spinal), using commercially available preparations or manipulated products.

Statistical analysis will be adopted to separately evaluate the effectiveness of interventions with single and multiple strains, and interventions with and without prebiotics.

Comparator(s)/ control

Comparators: only placebo interventions will be considered.

Other pharmacological interventions or no intervention will not be considered because these interventions comprise many comparisons that, if pooled all together, may not allow the assessment of the effectiveness of probiotics.

Context

Elderly people (aged 65 years or more) who live in the community or are institutionalized.

Unit of analysis issues:

The appropriate unit of analysis will be by individual, rather than hospital or centre. In studies with multiple intervention groups, we will include only groups that meet the eligibility criteria. If more than one intervention group of interest is identified, the analysis will be divided in to pair-wise comparisons.

Outcome(s)

Primary outcomes

Primary outcomes

- Prevention of infection: number of patients who did not develop an infection for each treatment group, together with the numbers of participants in each group;

- Incidence of adverse events: number of adverse events for each treatment group, together with the numbers of

participants in each group.

Secondary outcomes
Secondary outcomes

1) Prevention of emergency department visits: number of patients who did not seek for emergency department, among the patients who developed infections for each treatment group, together with the numbers of participants in each group;

2) Prevention of antibiotics use: number of patients who did not use antibiotics, among the patients who developed bacterial infections for each treatment group, together with the numbers of participants in each group;

3) Duration of antibiotic treatment: mean duration of antibiotic treatment, among the patients who developed bacterial infections and standard deviations for each treatment group, together with the numbers of participants in each group;

4) Duration of episodes of infection: mean duration of infection episodes, among the patients who developed infections and standard deviations for each treatment group, together with the numbers of participants in each group;

5) Duration of hospitalization due to infection: mean duration of hospitalization due to infection, among the patients who developed infections and needed hospitalization and standard deviations for each treatment group, together with the numbers of participants in each group;

6) Prevention of hospitalisation for infection treatment: number of patients who did not undergo hospitalization for infection treatment, among the patients who developed infections for each treatment group, together with the numbers of participants in each group;

7) Quality of life: effect on the perceptions of quality of life among the interventions groups, using generic or specific tools and scales for quality of life measurement, since already validated for each treatment group, together with the numbers of participants in each group;

8) All-cause mortality.

Data extraction, (selection and coding)
Selection of studies

For all the studies we identify from the electronic published resources and grey literature, two authors (PAW, PJFVB) will independently screen and review the titles and abstracts, and code them as "retrieve" (eligible or potentially eligible/unclear) or "do not retrieve" using a standardised form. Whenever necessary, chief investigators of selected trials will be contacted (through electronic contacts and/or address available in the manuscripts, or seeking updated their contacts through their institutions) to provide additional and relevant information regarding eligibility if it is unclear. Disputes regarding retrieving studies will be resolved by a third author (VSN).

We will retrieve all the full-text publications, and the authors previously mentioned (PAW, PJFVB) will independently screen the full-text and identify studies for inclusion, and record reasons for exclusion of the ineligible studies. Each trial´s reports will be scrutinized to ensure that multiple publications from the same trial are included only once. Disagreements about selection and inclusion/exclusion of studies will be resolved by consensus, and an independent reviewer (VSN) will be consulted if disagreement persists.

A reference management software will be used to manage the records retrieved from the searches, and to highlight duplications among the different databases. Any article in a language other than English will be translated. All data collected in the selection process will be used to complete a PRISMA flow chart and a table of `Characteristics of excluded studies´.

Data extraction and management :

Data will be extracted independently by two reviewers (PAW, PJFVB) using a standardised form, to minimize the

risk of error when extracting data, and to ensure that the same data is recorded for each study. This form will be pre-tested in a pilot. Whenever necessary, chief investigators of selected trials will be contacted to provide additional and relevant information, when it is not available in the full text manuscript.

Details of the study design, participant characteristics, characterization of the intervention(s), details on primary and secondary outcomes (randomised and analysed numbers in each treatment group), adverse effects and methodological quality (randomisation, blinding, allocation concealment, and assessment of risk of bias) will be extracted from each of the included studies.

The Quality Assessment Tool for Quantitative Studies, developed by the Effective Public Health Practice Project (EPHPP) will be used in conjunction, as a part of our data extraction form.

Dichotomous outcomes will be extracted as numbers of participants who experienced the outcomes in each group, and the total sample for both intervention and comparator. For continuous outcomes we will extract arithmetic means and standard deviations for each treatment group, together with the numbers of participants in each group.

If only standard error (SE) is reported, standard deviation(SD) will be calculated, as long as sample size is known, using SE = SD / square root of the sample size. Disagreements about extracted data will be resolved by consensus, and an independent reviewer (VSN) will be consulted if disagreement persists.

Risk of bias (quality) assessment

In order to assess the risk of bias in the included studies, two review authors (PAW, PJFVB) will independently evaluate the risk using The Cochrane Collaboration´s `Risk of bias´ tool (Higgins 2011). According to this tool, the following domains will be assessed:

1) random sequence generation;

2) allocation concealment;

3) blinding of staff, researchers and participants;

4) similarity of baseline outcome measurements;

5) similarity of baseline characteristics;

6) blinding of outcome assessment;

7) incomplete outcome data;

8) selective reporting;

9) other bias.

Each of these criteria will be explicitly judged by applying:

1) low risk of bias;

2) high risk of bias; or

3) unclear risk of bias (where unclear relates to the lack of precise information or uncertainty over the potential for bias).

Whenever necessary, chief investigators of selected trials will be contacted to provide additional and relevant information when they are not specified or unclear. Disagreements between the authors regarding the risk of bias will be resolved by consensus, and a third reviewer (VSN) will be consulted if disagreement persists.

Strategy for data synthesis

Measures of treatment effect:

Dichotomous data: results for dichotomous data will be presented as risk ratio with 95% confidence intervals.

Continuous data: for continuous data, we will use the mean difference, if outcomes are measured in the same way between trials. We will use the standardised mean difference to combine trials that measure the same outcome, but use different methods.

Dealing with missing data:

Whenever necessary, we will contact the chief investigators of clinical trials with missing data or unclear information (e.g. unclear risk of bias).

We will use intention-to-treat (ITT) analysis where the ITT sample consists of participants who, when randomised, took at least one dose of the assigned study intervention, and provided at least on post-baseline assessment.

Assessment of heterogeneity:

Heterogeneity will be assessed by visual inspection of the forest plot, and using both I-squared statistic and the Chi-squared test ($p<0.10$). I-squared values will be interpreted according to the current Cochrane Collaboration guidance (Higgins 2011), as follows:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

If the data are sufficiently similar we will perform quantitative synthesis by means of meta-analyses. If appropriate, we will perform meta-regressions.

Assessment of reporting biases:

We will search for protocols of included trials through PubMed and trial registries, when possible. We will contact study authors to attempt to establish full data or reasons for the non-reporting of specific outcomes. If there are sufficient trials (at least ten trials) we will construct a funnel plot to look for evidence of publication bias.

Data synthesis:

We will summarise data using the Cochrane Collaboration's statistical software, Review Manager 2013. We will decide on use of random- or fixed-effect models for data synthesis based on a number of factors. When significant clinical or methodological heterogeneity exists, we will use a random-effects model. Otherwise we will choose the fixed-effect model.

We will pool data using the relative risk for dichotomous outcomes and median difference for continuous outcomes. For random-effects meta-analysis we will use the Dersimonian and Laird method.

Where the data are too heterogeneous for combining effect sizes in a meaningful or valid manner, we will present the results of individual studies using a narrative approach in the review main text.

Analysis of subgroups or subsets

Subgroup analysis and investigation of heterogeneity:

We will identify heterogeneity statistically, through forest plot analysis, using I2 to determine if clinical heterogeneity might be a problem to combine trials results. When I2 is higher than 50%, we will consider high heterogeneity, and

low if less than 25%. If possible, we intend to perform subgroup analysis to consider the following:

a) age (young elderly (65-79 years) and very elderly (80 or older));

b) coexistence environment (institutionalised and elderly living in the community);

c) type of probiotic;

d) type of infection.

Sensitivity analysis:

Where appropriate, we will perform sensitivity analyses excluding trials with high or unclear risk of bias, and trials without full text information. Sensitivity analyses will also be performed to explore the differences between different models (fixed-effect versus random-effect) for pooling data where there is heterogeneity. Furthermore, we will perform this analysis for trials with imputed data.

Risk of Bias (RoB) Table:

For the risk of bias table the following outcomes will be considered:

• Prevention of infection;

• Survival;

• Prevention of hospitalisation for infection treatment;

• Prevention of emergency department visits;

• Prevention of antibiotics use;

• Quality of life;

• Incidence of adverse events.

Methods for rating the quality of evidence:

We will analyze the overall strength of the evidence for each outcome using the Grading of Recommendations Assessments, Developments and Evaluation (GRADE) (Balshem 2011; Guyatt 2008).

For each outcome that includes pooled data, we will analyse the following study limitations: inconsistency, imprecision, indirectness of evidence, risk of bias and potential publication bias. When appropriate, we will downgrade the evidence from `high quality´ by one level to ´serious´, or by two to `very serious´. Using the GRADE profiler software (GRADEPRO) we will create `Summary of findings´ tables, with the intention to provide outcome-specific information concerning the overall quality of evidence from studies included, and the magnitude of effect of the interventions examined (Purgato 2014).

Dissemination plans

After analysis and approval of this protocol record, we plan to publish the full text, to assure the readers and reviewers the access to it, ensuring the transparency of procedures in the publication of the systematic review.

We intend to publish the results of the systematic review, regardless of their findings, also in journal indexed, with impact factor greater than 3.0.

Contact details for further information

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Stage of review at time of this submission	Started	Completed
Preliminary searches	No	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Anexo 4

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Ética
Comitê de Ética em Pesquisa

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Ilustríssimo Senhor
Prof. Dr. Paulo José Fortes Villas Boas
Departamento de Clínica Médica da
Faculdade de Medicina de Botucatu

Prezado Prof. Paulo.

Informo que o Projeto de Pesquisa **"Probióticos para a prevenção de infecções em idosos: Revisão Sistemática da Literatura"**, a ser conduzido por Patrick Alexandre Wachholz, orientado por Vossa Senhoria, trata-se de revisão sistemática da literatura, portanto **não há necessidade de obtenção de parecer ético**.

Atenciosamente,

Prof. Dr.Trajano Sardenberg
Coordenador do CEP.