The function of probiotics on the treatment of ventilator-associated pneumonia (VAP): facts and gaps

Feride Karacaer,1 Imen Hamed,2 Fatih Özogul,3* Robert H. Glew4 and Dilek Özcengiz1

Abstract

Probiotics have been used for centuries in making fermented dairy products. The health benefits related to probiotics consumption are well recognized and they are generally regarded as safe (GRAS). Their therapeutic effects are due to the production of a variety of antimicrobial compounds, such as short-chain fatty acids, organic acids (such as lactic, acetic, formic, propionic and butyric acids), ethanol, hydrogen peroxide and bacteriocins. Ventilator-associated pneumonia (VAP) is a nosocomial infection associated with high mortality in intensive care units. VAP can result from endotracheal intubation and mechanical ventilation. These interventions increase the risk of infection as patients lose the natural barrier between the oropharynx and the trachea, which in turn facilitates the entry of pathogens through the aspiration of oropharyngeal secretions containing bacteria into the lung. In order to prevent this, probiotics have been used extensively against VAP. This review is an update containing information extracted from recent studies on the use of probiotics to treat VAP. In addition, probiotic safety, the therapeutic properties of probiotics, the probiotic strains used and the action of the probiotics mechanism are reviewed. Furthermore, the therapeutic effects of probiotic treatment procedures for VAP are compared to those of antibiotics. Finally, the influences of bacteriocin on the growth of human pathogens, and the side-effects and limitations of using probiotics for the treatment of VAP are addressed.

INTRODUCTION

Probiotics are non-pathogenic micro-organisms – in particular Lactobacillus and Bifidobacterium – that have beneficial effects on human health. Other such micro-organisms include Pediococcus, Lactococcus, Bacillus and yeasts. These micro-organisms contribute to the maintenance of intestinal microbial balance and enhance the host immune system. Probiotics are commonly found in dairy products such as yoghurt, milk and kefir, and also non-dairy products such as vegetables, fruits, fish and meat [1–3]. They are usually consumed by patients after receiving antibiotic therapy, and they can alter the normal microbial flora of the digestive tract (both the useful and the targeted harmful microbes). Probiotics are usually given the generally regarded as safe (GRAS) status [4]. However, since probiotics can have side-effects that include systemic infections, altered metabolism and gene transfer of antibiotic resistance from probiotic bacteria to pathogens, they should also be administered to immunodepressed individuals with care [5, 6].

Ventilator-associated pneumonia (VAP) is a common nosocomial infection in intensive care units (ICUs) and has a high morbidity rate. VAP is defined as any pneumonia that occurs 48–72 h following endotracheal intubation and mechanical ventilation (MV). It is characterized by signs of systemic infection accompanied by fever, altered white blood cell count and changes in sputum characteristics. VAP accounts for approximately half of all cases of hospital-acquired pneumonia [7, 8]. Early-onset VAP is less severe and occurs during the first 4 days of MV caused by antibiotic-sensitive bacteria (such as methicillin-sensitive Staphylococcus aureus, Streptococcus pneumoniae and Haemophilus influenzae). Late-onset VAP takes place 5 or more days after the beginning of MV caused by multidrug-resistant pathogens (Pseudomonas aeruginosa,
Acinetobacter, Enterobacter spp. and methicillin-resistant S. aureus), and is associated with increased mortality [9].

A variety of methods for preventing pneumonia have been proposed, including: reduction of the colonization of the aerodigestive tract by pathogenic bacteria, prevention of aspiration and limiting the duration of mechanical ventilation [10]. A novel intervention that has been proposed for treating VAP consists of administering probiotics that can prevent nosocomial infections in critically ill patients due to their prophylactic effects. In principle, probiotic therapy could provide a non-antibiotic strategy to reduce the risk of VAP [11, 12]. Although probiotic prophylaxis of VAP does not eradicate the pathogenic micro-organisms as antibiotics do, it can delay the time of bacterial colonization [13].

This paper reviews studies that have been conducted on the beneficial effects of probiotic administration in decreasing the rate of VAP and assesses the advantages and risks associated with probiotic therapy for VAP.

GENERAL INFORMATION ABOUT PROBIOTICS

The Greek word ‘probiotic’ means ‘for life’. According to the definition of the Food and Agriculture Organization and the World Health Organization (FAO/WHO), probiotics are ‘live micro-organisms, which when consumed in adequate amounts, confer a health benefit on the host’ [14]. Probiotic strains (Table 1) are usually represented by lactic acid bacteria (LAB), including Lactobacillus and Bifidobacterium. Non-pathogenic Escherichia coli, bacilli and yeasts such as Saccharomyces boulardii are also probiotics [15]. Several probiotic bacteria produce a variety of antimicrobial compounds, such as short-chain fatty acids, organic acids (lactic, acetic, formic, propionic and butyric acids), ethanol, hydrogen peroxide and bacteriocins (Fig. 1) [16, 17].

Probiotics are GRAS and capable of conferring health benefits. Perturbation of the intestinal microbiota can cause various chronic diseases, including functional bowel diseases, gastric ulcers, autoimmune diseases, colon cancer and cardiovascular disease.

Probiotic bacteria are capable of surviving passage through the digestive tract by virtue of their ability to resist gastric acid, bile salts and digestive enzymes, while they adhere to mucosal surfaces and colonize the intestine (at least temporarily), and proliferate in the gut because of their antimicrobial effects on potentially pathogenic bacteria. However, the ability of probiotic bacteria to remain viable in the gastrointestinal tract (GIT) and exert their beneficial effects needs to be documented for each strain [18, 19]. The criteria for use as a probiotic are shown in Fig. 2. Probiotics may affect other body sites in addition to the GI tract (such as the oral cavity, respiratory tract, urogenital tract and skin), and they can have applications in a variety of populations, including healthy individuals, children, the elderly, and immunocompromised and genetically predisposed individuals [20].

Lactobacillus species, Bifidobacterium species, Escherichia coli, Streptococcus species and Saccharomyces are frequently employed as probiotics [21]. Table 2 lists some of the commercially available probiotics and their preparations, route of administration and dosage.

Common causative pathogens of VAP include Gram-negative bacteria such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Acinetobacter species, and Gram-positive bacteria such as Staphylococcus aureus [22]. The causative pathogens for VAP are listed in Table 3.

THE THERAPEUTIC PROPERTIES OF PROBIOTICS AND THEIR SAFETY

Probiotics have a long history of use. Humans have been consuming them for many years along with fermented foods. However, the association between their intake and good health was only suggested relatively recently, at the beginning of the 20th century. In fact, it was not until the end of World War II (the so-called emerging antibiotics era) that probiotics were administered orally to treat or prevent diarrhoea associated with antibiotic use. Since then research regarding probiotic microbiology has progressed considerably and significantly [23, 24]. Scientific interest in probiotics is growing exponentially due to their numerous health benefits, ranging from improved lactose digestion to stimulation of the immune system, with the intention of invigorating health in general and preventing gastrointestinal (GI) cancer in particular [25]. Probiotics have been shown to be effective against three types of diarrhoea: antibiotic-associated diarrhoea, traveller’s diarrhoea and infectious diarrhoea [26]. In addition, probiotic bacteriocins and organic acids have been proven to protect the GIT by directly impeding the colonization of intestinal pathogens [27]. Lactase-deficient individuals encounter various degrees of abdominal discomfort (bloating, flatulence, nausea, diarrhoea and abdominal pain) after consumption of dairy products due to lactose malabsorption. Probiotic Lactobacillus acidophilus has been shown to alleviate the symptoms of lactose intolerance. Moreover, probiotics can stimulate the body’s immune response by enhancing humoral immune responses. The intestinal microflora is able to affect the development and functioning of the immune system. Probiotics may also stimulate the secretion of cytokines [28, 29]. Although many populations consume probiotics daily, some

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Table 1. Some of the micro-organisms used as probiotics

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Lactobacillus</td>
<td>Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus rhamnosus and Lactobacillus plantarum</td>
<td>[93]</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum and Bifidobacterium infantis</td>
<td>[93]</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Enterococcus faecalis and Enterococcus faecium</td>
<td>[18]</td>
</tr>
<tr>
<td>Escherichia</td>
<td>Escherichia coli Nissle 1917</td>
<td>[18]</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Saccharomyces boulardii</td>
<td>[55]</td>
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</table>
species constitute health risks that raise concerns about their therapeutic and commercial application [18]. Therefore, the safety of probiotics that are used industrially and commercially must be confirmed before they are marketed. Some of the problems concerning probiotic safety relate to the possibility of their causing illnesses, including bacteremia, fungaemia and endocarditis, or having a toxic influence on the intestinal system through the production of undesirable metabolites, or transferring antibiotic resistance to endogenous bacteria [6, 30]. The overall safety record of probiotics in humans is superb, especially for immunocompetent individuals. However, they should be used with caution in certain groups. For instance, probiotic sepsis has been reported in patients with deficient immune systems, GI disorders and chronic diseases, and in prematurely born neonates [5].

VENTILATOR-ASSOCIATED PNEUMONIA

VAP is the commonest hospital-originated infection in patients who require mechanical ventilation. The incidence of VAP differs depending on the patient group and the hospital setting, and it ranges from 6 to 52 per 100 cases. The VAP rate is usually 1 to 3% per day of intubation and mechanical ventilation [31]. The VAP-related mortality rate is between 24–76%, and is even higher in critically ill patients. VAP usually occurs 48–72 h after mechanical ventilation [32] and is related to the increased incidence of multidrug-resistant infections, increased antibiotic usage, extended mechanical ventilation time, patient stay in the ICU and patient stay in the hospital. Unsurprisingly, these factors increase the cost of patient management [33].

Endogenous flora is thought to play a central role in the pathogenesis of VAP, in particular, bacterial translocation from the stomach and oropharynx to the lower respiratory tract. Accumulation of oropharyngeal secretions by the endogenous flora occurs around the endotracheal tube cuff and microaspiration of these secretions is the main route of pathogenic invasion. Furthermore, pulmonary edema and previous lung infections facilitate bacterial proliferation [32].
It is difficult to diagnose VAP, since it cannot be defined by a single clinical manifestation. Usually chest radiology is used but, even if it is considered to be a very sensitive technique, it is typically nonspecific [34, 35]. Unfortunately, radiographic findings are not exactly correlated with pneumonia. The finding of alveolar haemorrhage or infarction, atelectasia and acute respiratory distress syndrome (ARDS) are unreliable in the diagnosis of pneumonia [36]. Clinical signs such as fever and leukocytosis, and pulmonary findings have intermediate predictive value. VAP has been diagnosed with new or advancing infiltration on chest X-rays and at least two of the factors: fever higher than 38°C, purulent secretions, leukocytosis, or leukopenia. However, the sensitivity was 69 % and the specificity was up 75 %, making its accuracy 72 % [37].

Because of its high morbidity and mortality, prevention of VAP is of great importance. Pharmacological and non-pharmaceutical VAP prevention strategies involve elevation of the bed’s head, subglottic secretion drainage, administration of antibiotics, selective digestive decontamination, chlorhexidine mouthwashes and lowering of the length of mechanical ventilation through constant use of sedation vacations and weaning protocols [11]. Probiotic therapy represents a non-antibiotic approach to addressing the patient’s aerodigestive bacterial balance and preventing VAP [38].

**ACTION OF PROBIOTICS’ METABOLITES ON VAP**

Healthy human GI system flora can support the gut barrier function by maintaining normal intestinal permeability. The normal GI flora of patients in an ICU is often replaced with pathogens. The use of broad-spectrum antibiotics may alter the normal gut microflora, which is important in preserving a healthy intestinal mucosal barrier. It is widely believed that impaired host immunity caused by pathogens has contributed to VAP in ICU patients receiving mechanical ventilation [39].

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Origin</th>
<th>Risk factor</th>
</tr>
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<tbody>
<tr>
<td>Methicillin-sensitive <em>Staph. aureus</em></td>
<td>Anterior nares</td>
<td>Traumatic coma, neurosurgical patients</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staph. aureus</em></td>
<td>Community-acquired</td>
<td>Prior antibiotic treatment, COPD, steroid treatment, longer duration of MV, prior bronchoscopy</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Oral cavity</td>
<td>COPD, early-onset VAP</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Lower GI tract</td>
<td>Antibiotic therapy, other infections</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Mouth, Skin, Lower GI tract</td>
<td>Antibiotic therapy, other infections</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td>Prolonged stay in hospital, prior antibiotic therapy, longer duration of MV, lung disease, late-onset VAP</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Upper respiratory tract</td>
<td>Aspiration, ARDS, neurosurgery poor hand-washing, late-onset VAP, corticosteroid therapy</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td>Oropharynx</td>
<td>Aspiration</td>
</tr>
<tr>
<td><em>Legionella</em></td>
<td>Water contamination</td>
<td>Chemotherapy, corticosteroid therapy, malignancy, renal insufficiency, neutropenia, contamination of (hospital) water system</td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
<td>Air flora</td>
<td>Corticosteroid therapy, cytotoxic drugs, COPD</td>
</tr>
<tr>
<td><em>Candida</em></td>
<td>GI tract</td>
<td>Immunosuppression, cytotoxic drugs, corticosteroid therapy, broad-spectrum antibiotics</td>
</tr>
<tr>
<td>Influenza</td>
<td></td>
<td>Winter season, immunosuppression, chronic underlying disease</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td></td>
<td>Immunosuppression, chronic cardiac or pulmonary disease</td>
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Goblet cells are distributed along the length of the intestines and produce mucins, which form the mucus barrier. Mucus is the first level of defense that intestinal bacteria encounter; pathogens must penetrate this barrier to reach the epithelial cells during infection. The mucus layer thins during inflammation, resulting in bacterial invasion and infiltration. Probiotic *Lactobacillus* species increase mucin production by human intestinal epithelial cells and block pathogenic *E. coli* invasion and adherence in *vitro* [40, 41].

Furthermore, probiotic bacteria enhance nonspecific defenses to microbial pathogens. Defensins and cathelicidins are peptides expressed constitutively by intestinal epithelial cells and display antimicrobial activity against a wide variety of bacteria, fungi and even some viruses [42]. The expression and secretion of select defensins are significantly upregulated in *vitro* by several commensal *E. coli* and *Lactobacilli* species [43]. Probiotics form antimicrobial substances such as short-chain fatty acids and bacteriocins, including microcins that can inhibit the growth of pathogens or kill them. Most probiotic bacteria synthesize acetic acid and lactic acid, which lower intestinal intraluminal pH and inhibit the growth of bacteria such as *Pseudomonas* and Enterobacteriaceae [44]. Wang et al. [45] demonstrated by meta-analysis that the application of probiotics deceased the risk of VAP caused by *P. aeruginosa* in 844 patients from 5 RCTs. Probiotics inhibit colonization of the stomach with the pathogen *P. aeruginosa*, which is acid-intolerant.

Probiotic bacteria have been shown to improve humoral immune responses, thereby protecting the intestinal immunological barrier. Probiotics can enhance the gut barrier function, which confers clinical advantages at distant sites on an immunomodulatory basis [46].

As a result, it has been suggested that probiotics decrease the incidence of VAP in critically ill patients by minimizing colonization by pathogens, optimizing host immune defenses, or both. These activities consist of minimizing the overgrowth of pathogens, improving gut barrier function, inhibiting pathogen adhesion, lowering bacterial translocation and up-regulating immune functions [47]. The mechanism for probiotics acting on VAP is shown in Fig. 3.

**THE EFFECTS OF PROBIOTICS ON VAP**

Due to multiple pathophysiological factors such as immune-system dysfunction or aerodigestive microflora change, ICU patients are particularly at risk of acquiring VAP. The use of probiotics to maintain or reconstitute normal aerodigestive microflora or prevent colonization by resistant bacteria has been proposed as a method to decrease VAP [48].

Knowing that VAP is usually caused by the aspiration of oropharyngeal pathogenic bacteria, Klarin et al. [49] investigated 50 mechanically ventilated patients, who were subjected to oral mechanical cleansing followed by washing with chlorhexidine (CHX) solution or oral application of an emulsion of *Lactobacillus plantarum* 299 (Lp299). When Lp299 was compared with standard oral chlorhexidine-based care, Lp299 was found to be safe and effective for oral care in intubated patients. However, there was no significant difference between CHX and Lp299 in the frequency of emerging pathogenic bacteria in the oropharynx and trachea.

Morrow et al. [11] administered a *Lactobacillus rhamnosus* GG count (2×10⁹ c.f.u) twice daily to patients requiring mechanical ventilation as a minimum of 72 h: 1×10⁹ c.f.u. *Lactobacillus* were given as a slurry to the oropharynx, while another 1×10⁹ c.f.u. *Lactobacillus* were administered through the nasogastric tube. In this study, the use of probiotic was related to a statistically significant decrease in the rate of VAP. *Lactobacillus* administration also led to a crucial delay in the beginning of microbiologically diagnosed VAP. In addition, probiotic use resulted in a statistically significant decline of *C. difficile* incidence associated with diarrhoea, and antibiotic consumption for *C. difficile*.

Knight et al. [50] enterally administered symbiotic therapy or a placebo to 259 heterogenous critically ill patients in the RCT who were receiving mechanic ventilation. Oropharyngeal throat swabs were obtained on admission to the trial and on the 4th and 7th days. The isolated bacterial strains and the rates of colonization by pathogens did not change with the use of symbiotic therapy, which did not reduce the incidence of VAP or the duration of mechanical ventilation.

Forestier et al. [51] examined the effect of *Lactobacillus casei rhamnosus* on gastric and respiratory tract colonization and infection with *Pseudomonas aeruginosa*, which is a frequently isolated antibiotic-resistant Gram-negative organism in VAP. In this study, the emergence of *P. aeruginosa* respiratory colonization and/or infection was delayed significantly in the probiotic group compared to the placebo group.

Siempos et al. [52] conducted a meta-analysis that examined the effects of probiotics on VAP. This included 5 RCTs (2 of which were discussed above in [49–51]) and a total of 689 patients. They concluded that the incidence of VAP was significantly lower in patients undergoing MV treated with probiotics compared to controls. In addition, probiotics were associated with a shorter ICU stay and lower colonization of the respiratory tract with *Pseudomonas aeruginosa*, but not with lower mortality from all causes or shorter MV duration compared to control.

Petrof et al. [53] investigated the effects of probiotics in critically ill patients in a meta-analysis that included 23 RCTs and 2153 patients. The results showed that probiotics were associated with a reduction in infectious complications, including VAP, and a trend toward decreased ICU mortality. However, probiotics had no effect on in-hospital mortality, length of stay in the ICU or hospital, or diarrhoea.

Bo et al. [46] conducted a meta-analysis that examined the effects of probiotics on VAP and included 8 RCTs and 1083 patients [11, 50, 51]. Their main finding was that the incidence of VAP declined to a statistically significant degree in patients receiving probiotics. In addition, the length of
antibiotic use (days) was reduced in patients receiving probiotics. However, there was no significant difference in ICU mortality, in-hospital mortality, length of ICU stay, or duration of mechanical ventilation.

In contrast to these findings, in a meta-analysis that included 844 patients from 5 RCTs, Wang et al. [45] concluded that probiotics did not significantly decrease the incidence of VAP. Although the probiotic group did show a trend towards lower incidence of VAP, the prophylactic effect was not significant. In addition, the mortality and length of stay in the ICU and hospital were not reduced. However, *P. aeruginosa* infection was found to decrease.

The incompatible results between studies may be due to differences in patients and clinical situations, the method of probiotic administration, the criteria used in the diagnosis of VAP and the sample size, or the heterogeneity of the probiotic strains used. We conclude that there is insufficient evidence of efficacy and safety to warrant routine the application of probiotics for the prevention of VAP in critically ill patients. Multicentre, large-scale, randomized trials of probiotics and VIP are needed.

**TREATMENT PROCEDURES FOR PROBIOTICS AND VAP**

Despite conflicting results in RCTs, prophylactic use of probiotics can be a preventive method for VAP. RCTs employing a variety of probiotic formulations and administration methods have provided useful information regarding the role of probiotics in treating VIP. In most studies, probiotics alone were delivered into the stomach via nasogastric or orogastric tubes [13]. Probiotics have also been introduced into the mucosal surface of the oral cavity [49, 51]. For example, Morrow et al. [11] administrated *Lactobacillus rhamnosus* to the oral mucosa and the stomach by means of nasogastric tubes. This combined delivery strategy decreased the incidence of VAP and led to fewer days of antimicrobial therapy.

Although there is no consensus regarding the timing of the administration of probiotics, several RCTs have suggested that probiotics should be used shortly after the onset of mechanical ventilation. The length of probiotic treatment changes based on indication. When being used for the prevention of VAP, probiotics should be administered until the risk factor is removed. In clinical trials the median treatment duration for probiotics has been 14–21 days [21].

Although dose response data are lacking for adults, a recent systematic review in a paediatric population demonstrated that probiotic doses of 5 billion colony-forming units (c.f.u.)/day or greater were more effective than lower doses in reducing diarrhoea [54]. According to this result, Petrof et al. [53] analysed a subgroup in their meta-analysis and found that that rates of infectious complications were similar between trials using high-dose probiotics ($\geq 5 \times 10^9$ c.f.u. day$^{-1}$) and trials using a lower dose of probiotics ($<5 \times 10^9$ c.f.u. day$^{-1}$) [55].

**USE OF PROBIOTICS COMPARED TO ANTIBIOTICS FOR VAP**

Topical application of non-absorbable antibiotics is a strategy for the prevention of colonization of the upper and/or lower digestive tract. Selective decontamination of the
digestive tract (SDD) and selective oropharyngeal decontamination (SOD) have been used in clinical studies for the prevention of VAP [56]. SDD aims to eliminate potential pathogenic micro-organisms (PPM) in the oral cavity, stomach and intestines by means of the application of non-absorbable antibiotics. In general, tobramycin, colistin and amphotericin B are used as oropharyngeal paste and enteral antibiotics, while a second-generation cephalosporin is used as an intravenous antibiotic in the first 4 days of ICU admission [57].

The aim of SOD is to eliminate PPM in the oral cavity by the local application of non-absorbable antibiotics [13]. Schnabel et al. [58] reviewed the incidence of VAP before and after the onset of SOD alone and SDD in general ICU patients. SOD was administered as the standard of care in December 2010 and SDD, including SOD, was administered in January 2012 for all patients with an expected length of ICU stay of at least 48 h. The incidence of VAP per 1000 ventilator days decreased from 4.38±1.64 before therapy to 1.64±0.43 after the introduction of SOD/SDD.

Bo et al. [46] investigated the impact of SDD on hospital-acquired infections in ICU patients, and specifically on the duration of MV in Guillain–Barré syndrome (GBS). In this study, SDD reduced the length of ventilator time for mechanically ventilated GBS patients, probably by preventing VAP. In another study, 271 patients who were admitted to the ICU and were expected to require intubation for in excess of 48 h were enrolled and received topical antibiotics or a placebo. Uninfected patients additionally received ceftriaxone or a placebo for 3 days. VAP incidence was significantly lower in the SDD group compared to the control group. Furthermore, non-respiratory infections, length of ICU stay and mean cost were lower in the SDD group relative to the controls [59]. Most studies have documented reductions in the incidence of VAP with SDD. However, in most of these studies, SDD concentrations and dosing frequencies, patient populations, use of other antibiotics and feeding regimens varied – as did the diagnostic criteria [13].

Probiotics have also shown preventative effects against VAP in multiple meta-analyses, as mentioned above [46, 52, 53]. However, other meta-analyses have concluded that the use of probiotics confers no benefit [45, 59]. Studies of probiotics were also as heterogeneous as studies of antibiotics. While probiotics alone are not expected to eradicate the PPM as antibiotics would do, they may delay bacterial colonization [13].

The development of resistant bacteria is a widely acknowledged risk associated with prophylactic antibiotic use. In fact, most studies of SDD/SOD have not demonstrated the occurrence of resistant bacteria. Krueger et al. [60] administered SDD (intravenous ciprofloxacin with a mixture of topical gentamicin and polymyxin applied to the nostrils, mouth and stomach for 4 days) or a placebo. They did not find notable differences between the two groups with regard to the isolation of resistant bacteria. Infections with Gram-negative bacteria (GNB) resistant to ciprofloxacin occurred in 5 of the 265 patients in the SDD group, and in 7 of the 262 patients in the placebo group. Infections with GNB that were resistant to gentamicin occurred in 4 patients from the SDD group and in 10 patients from the placebo group. Infections with GNB that were resistant to polymyxin appeared in 2 patients from the SDD group and in 18 patients from the placebo group.

A multicentre cluster-randomized crossover trial compared SDD, SOD and standard care (no SDD or SOD) for 5939 patients in 13 Dutch ICUs for 6 months per intervention. Participants admitted to ICUs with an expected duration of mechanical ventilation of more than 48 h or an expected stay of more than 72 h received SOD (topical tobramycin, colistin and amphotericin B in the oropharynx), SDD (SOD antibiotics in the oropharynx and stomach plus 4 days of intravenous cefotaxime), or standard care. In this study, ICU-acquired bacteraemia with highly resistant microorganisms (mainly GNB) emerged less often in the SDD group than in the SOD and standard care groups [crude odds ratios (95% confidence intervals): SDD versus SOD, 0.37 (0.16 to 0.85); SDD versus standard care, 0.41 (0.18 to 0.94); SOD versus standard care, 1.10 (0.59 to 2.07)]. Endotracheal aspirate cultures were obtained from patients who stayed longer than 3 days in ICUs. The respiratory tract colonization with Gram-negative bacteria or cefotaxime-resistant and colistin-resistant pathogens was lowest in the SDD group [61]. The results from large SDD/SOD trials in settings with low levels of antibiotic resistance strongly suggest that SDD and SOD can be used safely in the treatment of ICU patients.

However, the use of SDD and SOD was shown to affect the bacterial ecology, with rising ceftazidime resistance prevalence rates in the respiratory tract during intervention and a notable rebound effect of ceftazidime resistance in the intestinal tract after discontinuation of SDD [62]. In addition, SDD and SOD are not effective against resistant Gram-positive bacteria and the use of SDD/SOD may facilitate colonization with Gram-positive pathogens such as Staph. aureus [13].

Oudhuis et al. [63] administrated SDD four times per day every day in different forms as an oral paste (polymyxin E, gentamicin and amphotericin B) and an enteral solution (the same antibiotics). Intravenous cefotaxime was used during the first 4 days. LAB (L. plantarum 299/299 v) and rose-hip were also applied twice daily to 254 patients who were expected to receive mechanical ventilation within/after 48 h and/or had a foreseeable ICU stay of 72 h or more. The acquired infection incidence was 28% (70/254); 40/130 participants in the LAB group (31%) and 30/124 participants in the SDD group (24%). There was no significant difference between the groups, but there was a trend towards more infections in the LAB group. The ICU mortality rate did not differ between the groups (OR 0.99, 95% CI 0.51–1.92; P=0.97). There was no increase in antibiotic resistance during or after use of SDD and LAB. Thus, these
investigators could not prove that the combined effect of *Lactobacillus plantarum* 299/299v and rose-hip was less effective than SDD in preventing infection in standard ICU patients.

Several other trials have examined probiotics’ efficacy in the prevention of VAP, as mentioned above. According to these studies, probiotics are safe and effective for oral care in intubated patients [49], and are associated with a statistically important decline in the incidence of VAP and a trend towards decreased ICU mortality [11, 46, 52, 53]. Although there are other studies with different results [45], prophylactic use of probiotics seems to be beneficial strategy for VAP. One concern regarding probiotic use is the risk of diarrhoea in critically ill patients. However, several trials have reported that the incidence of diarrhoea did not differ between participants receiving probiotics and those not receiving them [50, 63, 64]. Another concern with regard to the safety of probiotics is the fact that adherence to the intestinal mucosa may also increase bacterial translocation and virulence. The most potent probiotics, therefore, may have increased pathogenicity [5]. Therefore, another area of concern for probiotic use is the risk of secondary infections. Although to date bacteremia and fungemia have not been reported in studies on probiotic use, there are case reports implicating probiotics in endocarditis and bacteremia [65]. In addition, a probiotic trial in pancreatic patients was terminated because of increased mortality rates in patients administered with probiotics [66].

SDD and SOD have been shown to prevent VAP, but prophylactic use of antibiotics may cause the bacterial environment to change, with increased ceftazidime-resistance strains in the respiratory and intestinal tracts. Probiotic prophylaxis of VAP is still uncertain and the risks and benefits should be assessed differently according to each patient. Additional SDD/SOD and probiotic trials are needed in order to understand the exact advantages and disadvantages of probiotics and antibiotics for the prevention of VAP.

**THE INFLUENCE OF BACTERIOCIN ON HUMAN PATHOGEN GROWTH CAUSED BY VAP**

Certain bacteriocins can inhibit the growth of pathogenic bacteria. Bacteria that lack the bacteriocin gene produce less potent probiotic action. Bacteriocins have wide-spectrum effectiveness against Gram-positive and Gram-negative bacteria, as well as yeasts and moulds [67]. The non-pathogenic oral organism *Streptococcus salivarius* is one of the major bacteriocin producers that is able to reduce the frequency of colonization of the upper respiratory tract by pathogens involved in infections [68]. The lantibiotic salivaricin D produced by the probiotic *S. salivarius* strains can inhibit Gram-positive pathogens of the upper respiratory tract, such as *S. pneumoniae* or *S. pyogenes* [69]. Salivaricin-producing *S. salivarius* is effective against other human pathogens, including *Moraxella catarrhalis* and *Haemophilus influenzae* [70]. *Lactobacillus crispus* suppressed the growth of *Haemophilus influenzae* [71]. Bacteriocins isolated from *Lactobacillus species* (*L. fermentum* and *L. casei*) were tested against several pathogens, including *Staphylococcus aureus, Klebsiella pneumonia* and *Haemophilus influenzae*, and they were found to have a broad range of activity against selected pathogenic bacteria [72].

**SIDE-EFFECTS OF PROBIOTICS**

In addition to the widely recognized beneficial health effects of probiotics, complications associated with their consumption (endocarditis, antibiotic resistance, lactobacilllum, bifidobacteremia and fungemia) appear to be rare [73, 74]. Lactobacilli are generally considered to be non-pathogenic. However, they can cause serious infections, including bacteremia, endocarditis, meningitis, pleuropulmonary infection, intra-abdominal abscesses, urinary tract infections and dental caries [75, 76]. The use of probiotics can be toxic in the GIT, since they can produce undesirable metabolites (conjugated bile acid), especially in patients with short small bowel syndrome. The conjugated bile acid metabolites might accumulate and lead to malabsorption [30]. This might increase the risk of colon cancer. A case of aortic valve endocarditis caused by *Lactobacillus casei* presented in a 53-year-old immunocompetent patient with a past history of rheumatic fever [77]. Other cases were described regarding *Lactobacillus* probiotic-associated endocarditis for patients with clinical histories [78, 79]. Usually the infections caused by lactobacilli can be correlated with previous illnesses, such as recent surgery, transplants, valvulopathy, diabetes mellitus, AIDS and cancer [80]. Bacteremia has been observed in a variety of clinical settings in which the following probiotic bacteria were the causative agent: *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus delbrueckii, Lactobacillus acidophilus* and *Bifidobacterium longum* [76, 81–84]. Numerous cases of fungemia due to *Saccharomyces cerevisiae* subtype *boulardii* have been reported [85–87]. Antibiotic-resistant probiotics could be used to restore the gut microflora following antibiotic treatment. However, the transfer of resistant genes to pathogenic bacteria represents a serious clinical threat, since pathogens would be protected against commonly used drugs [88]. Thus the existence of antibiotic resistance determinants in the genome of probiotic bacteria have to be thoroughly tested and care should be taken when selecting probiotic strains [89, 90]. The side-effects of the probiotics are summarized in Fig. 4.

Even if the complications cited above are rare, probiotics should not be taken by patients with fragile health who are immunocompromised or critically ill. Case studies reporting negative effects related to probiotic use were described among health-compromised rather than healthy individuals [91].

**ADVANTAGES AND DISADVANTAGES OF PROBIOTICS FOR THE TREATMENT OF VAP**

Probiotics have been shown to beneficially affect digestive function and enhance the immune system. Probiotics have...
often been used to provide prophylaxis against pathogens. In fact, they have been shown to possess antimicrobial and anti-inflammatory effects that are important for some people, especially critically ill patients with a variety of disease states [92]. In addition, they have several advantages, such as ease of administration, low cost and minimal toxicity [46]. Because of the potential risks associated with probiotic use, these beneficial effects have occasionally been underestimated or overlooked. These risks include increase of antimicrobial resistance, increase of bacterial translocation, iatrogenic infection and diarrhea. Despite the obvious benefits of probiotics in ICU patients, current use by most clinicians is limited due to the aforementioned risks [21]. Table 4 shows the benefits and drawbacks of probiotic use for treatment of tVAP.

CONCLUSION

VAP usually develops more than 48 h after endotracheal intubation and is an important cause of in-hospital morbidity and mortality, and increased length of hospital stay and higher medical costs. Prevention of colonization of the upper and lower aerodigestive tract is one approach for preventing VAP. Recently, probiotics have been used for this purpose. It is widely believed that probiotic bacteria can decrease the development of VAP through local and systemic actions that improve GI barrier function, increase host cell antimicrobial peptides, regulate the composition of the intestinal flora, and reduce overgrowth of pathogenic bacteria and bacterial translocation. Several studies have demonstrated that probiotics can be safe and efficacious in preventing or ameliorating VAP in ICU patients. However, unsurprisingly, since the designs and methodologies of those studies were heterogeneous in terms of ICU population, number of subjects, varying pneumonia diagnoses, the nature of the probiotic product used, and differing dosing and treatment periods, consistent and definitive generalizations cannot be drawn. Therefore, although probiotics are usually considered to be safe and well tolerated, wide, well-planned, randomized and multicentre studies are required to verify the efficacy of probiotics against VAP before they can be recommended for routine clinical application.

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References