Probiotics for the Prevention of Ventilator-Associated Pneumonia: Belle of the Ball or Premature Call?

Learning Objectives:
1. Define ventilator associated pneumonia and identify its related complications
2. Discuss current preventive measures for ventilator associated pneumonia
3. Describe the various mechanisms of probiotics
4. Evaluate the current literature for the use of probiotics for the prevention of ventilator associated pneumonia
Ventilator-Associated Pneumonia

1. **Definition**\(^1,2\)
   a. Pneumonia is acute infection of the pulmonary parenchyma
   b. Ventilator-associated pneumonia (VAP) is pneumonia that occurs more than 48-72 hours after endotracheal intubation

2. **Epidemiology**\(^2,3\)
   a. Incidence
      i. Second most common nosocomial infection in the United States
      ii. Occurs in up to 30% of patients who are mechanically ventilated

3. **VAP Complications**\(^4-7\)
   a. Death (27-43% mortality rate)
      i. Twice as likely to die compared to those who do not develop VAP
   b. Prolongation of mechanical ventilation (MV) by 4 days
   c. Increase ICU length of stay (LOS) by 5-7 days
   d. Increase hospital LOS by 2-3 fold
   e. Increase costs ($40,000 per hospital admission)
   f. Necrotizing pneumonia with pulmonary hemorrhage
      i. Bronchiectasis and parenchymal scarring leading to recurrent pneumonias
   g. Muscle loss, general debilitation, and prolonged rehabilitation
      i. Inability to return to independent function
      ii. Nursing home or long term care placement

4. **Risk Factors**\(^4,7-9\)
   a. Endotracheal tube
      i. Tube with concomitant need for suctioning may damage mucosa; facilitating colonization
      ii. Eliminates coughing reflex- mucociliary clearance is impaired
   b. Biofilm
      i. Creates a barrier that protects microorganisms from host defenses and antibiotics
      ii. As early as 12 hours after intubation, biofilm contains large amounts of bacteria that can be disseminated into the respiratory tract with ventilator induced breaths
   c. Enteral feedings
      i. Increase gastric pH- increases risks for bacterial colonization
      ii. Increase gastric volume- increases risks for aspiration
   d. Stress ulcer prophylaxis
      i. Increased gastric contents and pressure on the lower esophageal sphincter- retrograde movement of gastric contents up to the esophagus
      a. Subsequent aspiration of gastric content and bacteria
   e. Patient population
      i. Severely ill patients with sepsis or trauma
      ii. Prior antibiotic exposure (at higher risk for multi-drug resistant pathogens)
      iii. Immunosuppression, chronic obstructive lung disease, and acute respiratory distress syndrome
      iv. Age
         1. Elderly residents of long term care facilities have been found to have a spectrum of pathogens that most closely resembles late-onset VAP
5. **Etiology**

<table>
<thead>
<tr>
<th>Common pathogens with early onset VAP (&lt; 5 days)</th>
<th>Common pathogens with late onset VAP (≥ 5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Methicillin-sensitive Staphylococcus aureus</em></td>
<td><em>Acinetobacter</em> spp.</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Enterobacter</em> spp.</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Legionella pneumophila</em></td>
</tr>
</tbody>
</table>
| *Proteus* spp. | |}

a. Less commonly associated with fungal or viral pathogens  
   i. Usually affects severely immunocompromised patients
b. Late onset VAP (≥ five days) are more likely to be caused by multi-drug resistant pathogens¹ (see Appendix A)  
   i. Associated with higher morbidity and mortality

6. **Pathogenesis**

a. Balance between host defenses and microbiological propensity for colonization shift in favor of the ability of pathogens to persist and invade the lower respiratory tract¹
b. It remains unknown which anatomic sites are the most important targets for modifying the host flora with probiotic therapy

   | Microaspiration of contaminated secretions |
   | Colonization of aerodigestive tract with pathogenic bacteria |
   | Formation of biofilms |
   | Leakage of bacteria around the endotracheal cuff into the lower respiratory tract |
   | Stomach and sinuses may serve as potential reservoirs for colonization of pathogens |

   i. Increase risk of gastrointestinal reflux, provides a route for bacteria to translocate to the upper airway

7. **Diagnosis of VAP**

a. Pneumonia that arises greater than 48 hours after intubation
b. Clinical approach  
   i. Radiographic infiltrate and at least two clinical features  
      1. Fever > 38°C  
      2. Leukocytosis or leucopenia (< 4,000 cells/mm³ or > 12,000 cells/mm³)  
      3. Purulent tracheal secretions  
   ii. Clinical pulmonary infection scoring (see Appendix B)  
      1. Combines clinical, radiographic, physiological and microbiological data into a single numerical result  
      2. Look at temperature, white blood cell count, tracheal secretion, PaO₂/FiO₂, and chest x-ray  
      3. Score > 6; good correlation of pneumonia  
      4. Re-evaluate at day 3; may indicate safe discontinuation of antibiotic if score < 6
c. Bacteriologic approach
   i. Cultures:
      1. Endotracheal tube: > 10^6 cfu/mL
      2. Bronchoalveolar lavage: > 10^4 or 10^5 cfu/mL
      3. Protected brush specimen: > 10^3 cfu/mL
   ii. Signs of clinical response
       1. Temperature, WBC, chest x-ray, oxygenation, purulent sputum, hemodynamic changes, and organ function

Figure 1. Diagnostic approach

8. Current prevention of VAP^3,10-15
   a. Infection control measures
      i. Staff education
      ii. Compliance with alcohol-based hand disinfection
      iii. Isolation to reduce cross-infection of MDR pathogens
Table 2- Pathogenic mechanisms and corresponding prevention strategies

<table>
<thead>
<tr>
<th>Pathogenic Mechanism</th>
<th>Prevention Strategy</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroesophageal reflux</td>
<td>Avoid high gastric residuals; prokinetic agent</td>
<td>Implementation of a VAP bundle in a hospital facility has shown to decrease VAP incidence by as much as 3.5 cases per 1000 ventilator days. However, compliance with hospital infection protocol often wanes with time and can be significantly influenced by staff in the ICU.</td>
</tr>
<tr>
<td>Bacterial overgrowth of stomach</td>
<td>Limit use of stress ulcer prophylactic agent; selective decontamination</td>
<td></td>
</tr>
<tr>
<td>Large-volume aspiration</td>
<td>Avoidance of sedation</td>
<td></td>
</tr>
<tr>
<td>Endotracheal intubation</td>
<td>Minimize duration; use of non-invasive ventilation</td>
<td></td>
</tr>
<tr>
<td>Secretions pooled above endotracheal tube</td>
<td>Elevate head of the bed (30-45°) (34% reduction in VAP incidence); subglottic suctioning; avoidance of reintubation; cuff pressure maintained at 20-25 cm H2O</td>
<td></td>
</tr>
<tr>
<td>Prolonged duration of ventilation</td>
<td>Daily awakening; sedation vacation; weaning protocols (Decrease mechanical ventilation by 2 days; decrease ICU LOS by 3.5 days)</td>
<td></td>
</tr>
<tr>
<td>Aspiration</td>
<td>Intensive oral care every 6-8 hours and as needed, in addition to chlorhexidine mouth wash (up to a 26% reduction in VAP incidence)</td>
<td></td>
</tr>
</tbody>
</table>

**Probiotics**

9. **History of Probiotics**

a. The term probiotic literally means “for life”
   i. Coined in the 1960s by Lilly DM et al.

b. Probiotics effects extend beyond the gastrointestinal system and offer benefits to disorders of the respiratory tract, urogenital system, oral health, and allergic diseases

c. Early study by Gluck et al.
   i. 209 volunteers randomly assigned to probiotic (*Lactobacillus GG, Bifidobacterium, Lactobacillus acidophilus, and Streptococcus thermophilus*) or placebo
   ii. Nasal microbial flora analyzed
      1. Significant reduction in occurrence of nasal pathogens with probiotic versus placebo (*p* < 0.001)
      iii. Regular intake of probiotics can reduce nasal pathogens in the upper respiratory tract

d. With increasing ICU antibacterial resistance rates and fewer new antibiotics in the research pipeline, focus has shifted to non-antibiotic approaches for the prevention and treatment of nosocomial infections

Table 3- Other proposed uses for probiotics

<table>
<thead>
<tr>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic associated diarrhea</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> infections</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
</tr>
<tr>
<td>Multi-organ dysfunction syndrome</td>
</tr>
<tr>
<td>Allergies (eczema)</td>
</tr>
<tr>
<td>Inflammatory bowel disease and bowel syndrome</td>
</tr>
</tbody>
</table>
Table 4 - Commercially available products

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florastor™</td>
<td><em>Saccharomyces boulardii</em></td>
</tr>
<tr>
<td>Align™</td>
<td><em>Bifidobacterium infantis</em></td>
</tr>
<tr>
<td>DanActive™</td>
<td><em>Lactobacillus casei</em></td>
</tr>
<tr>
<td>Activia™</td>
<td><em>Bifidobacterium lactis</em></td>
</tr>
<tr>
<td>Culturelle™</td>
<td><em>Lactobacillus rhamnosus GG</em></td>
</tr>
<tr>
<td>Floranex™</td>
<td><em>Lactobacillus acidophilus</em></td>
</tr>
<tr>
<td>Lactinex™</td>
<td><em>Lactobacillus acidophilus and Lactobacillus helveticus</em></td>
</tr>
</tbody>
</table>

10. Probiotics versus Prebiotics versus Synbiotics

a. Probiotics
   i. Living microbial agents of human origin that are able to tolerate the hostile gastrointestinal environment (acid and bile) such that they ultimately persist in the lower alimentary tract to confer health benefits to the host
   ii. World Health Organization (WHO) has defined probiotics as “live microorganism which, when administered in adequate amounts, confer a health benefit on the host”

b. Prebiotics
   i. Non-digestible ingredients that stimulate the growth and activity of bacteria in the gut
   ii. Selectively promote the growth or activity of beneficial bacteria

c. Synbiotics
   i. Combination of probiotics and prebiotics
      1. Designed to improve the survival of ingested microorganisms and promote colonization of the intestinal tract

11. Proposed Mechanisms of Probiotics

a. Probiotics become part of the established intestinal microflora
b. Temporarily remodel or influence the microbial community
c. Barrier function
   i. Goblet cells produce a thick layer of glycosylated mucoproteins, called mucin
      1. Forms a mucus barrier; acts as first line of defense for intestinal bacterial
      2. Prevent damage, repair, and restore the mucosal integrity
      3. During infection and inflammation
         a. Mucus layer is markedly thinner, allowing bacterial adherence and infiltration
      4. Probiotics increase mucin expression in human intestinal cells and block pathogenic invasion and adherence in vitro

d. Epithelial adherence
   i. Probiotics compete with invading pathogens for binding sites to the epithelial cells and the overlying mucus layer
   ii. Secretion of a non-bacteriocin component that acts on the pathogen or host cell to inhibit adherence of various pathogens

e. Host cell antimicrobial peptides
   i. Defensins and cathelicidins are peptides expressed by intestinal epithelial cells
      1. Display antimicrobial activity
   ii. Expression and secretion of select defensins are significantly upregulated with *Lactobacillus* species exposure
   iii. Panteth cells, localized at the base of epithelial cell crypts
1. Produce a variety of peptides with antimicrobial properties

f. Probiotic antimicrobial factors
   i. Directly inhibit the growth or killing of pathogens by production of antimicrobial molecules
      1. Short chain fatty acids
         a. Disrupts the outer membrane of gram negative bacteria
      2. Bacteriocins (bactericidal proteins)
         a. Decrease luminal pH - inhibits the development and colonization of pathogens
         b. Inhibit bacterial adhesion to epithelial cells
         c. Increase permeability of the inner membrane of gram negative bacteria
            i. Leads to disruption of cell wall synthesis and porin formation
   3. Microcins
      a. Interfere with enzymes involved with DNA, RNA, or protein synthesis
      ii. Lower intestinal intraluminal pH which inhibits the growth of pathogens

g. Immunomodulation
   i. Induce regulatory T cells that act as a brake on the effector T cells (that would normally cause inflammation)
   ii. Act on toll like receptors on epithelial cells
      1. Induce the production of protective cytokines, such as IL-6, that mediate epithelial cell regeneration and inhibit epithelial cell apoptosis
      2. Induce the downregulation of certain inflammatory markers including protein kinase C, IL-8 and IL-6
   iii. Block cytokine induced upregulation of multiple cell signaling gene expression
      1. Halts cytokine signaling

h. Effects on lymphoid cells
   i. Induce macrophages to express increase amounts of nitric oxide and inflammatory cytokines
      1. Exhibit increase anti-viral activity
   ii. Produce increased amounts of granulocyte-colony stimulating factors
      1. Compared to subjects exposed to pathogenic *E. coli*
   iii. Increase natural killer cell activity
   iv. Increase elaboration of regulatory cytokines

12. Regulation of probiotics
    a. Dependent on the intended use of a probiotic, regulatory requirements differ
       i. Food and Drug Administration (FDA)
       ii. Center for Food Safety and Applied Nutrition
    b. FDA defines a drug as an article intended “for use in the diagnosis, cure, mitigation, treatment or prevention” of a disease
    c. Dietary Supplement Health and Education Act (DSHEA) defines a dietary supplement as “a product taken by mouth intended to supplement the diet”
       i. Does not have to be FDA approved before marketed to the public
       ii. The manufacturer is responsible for determining the safety and purported claims of probiotics
    d. In 2001, WHO proposed guidelines to standardize the health claims regarding probiotics
       i. Delineate the mechanism of the probiotic effect
       ii. Substantiate its clinical health benefits
       iii. Safety assessment
13. Recap
   a. Though preventive measures exist for preventing VAP, it remains one of the most common hospital acquired infections
   b. Other preventative measures exist and definitively target the pathogenic mechanism of VAP
      i. No true understanding probiotics’ mechanism of action
      ii. No true anatomic target of probiotics role

Literature Review

14. Meta-Analyses (see Appendix C, D)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective</strong></td>
<td>To evaluate the efficacy of probiotics for the prevention of VAP in adult patients undergoing mechanical ventilation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Study Design** | • Studies published between 2006- 2009  
• Sample size ranged from 50 to 300 patients  
• 5 randomized controlled trials included |
| **Inclusion Criteria** | • Adult inpatients undergoing mechanical ventilation  
• Probiotics compared to control  
• Data available on the incidence of VAP |
| **Exclusion Criteria** | • Studies that did not specifically refer to VAP  
• Studies that did not provide original data  
• Studies that had probiotics in both arms |
| **Outcomes** | • Primary: incidence of VAP  
• Secondary: all-cause mortality, ICU length of stay, duration of mechanical ventilation (until patient’s death or extubation), colonization of respiratory tract with *Pseudomonas aeruginosa*, diarrhea, and probiotic-induced bacteremia and fungemia |
| **Methods** | • 5 randomized controlled trials included with a total of 689 patients  
• 2 of 5 studies tested probiotics *Lactobacillus plantarum* 299 and *Lactobacillus casei rhamnosus*  
• 3 of 5 studies tested Synbiotic 2000FORTE  
• Probiotics were administered via the nasogastric or orogastric tube  
• Probiotics were administered BID in the three of the studies (2- administered once daily) |
| **Statistical Analysis** | • Analyzed as odds ratio with 95% confidence intervals  
• I² statistic for heterogeneity |
| **Results** | • Baseline characteristics: patients >16 years of age requiring mechanical ventilation > 24 hours  
• Primary outcome: VAP incidence (18.9% vs. 27.3%) |

<table>
<thead>
<tr>
<th>OR 95% CI</th>
<th>I² ,%</th>
<th>p-value for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.61 (0.41-0.91)</td>
<td>39</td>
<td>0.16</td>
</tr>
</tbody>
</table>

• Secondary outcomes:

<table>
<thead>
<tr>
<th>OR 95% CI</th>
<th>I² ,%</th>
<th>p-value for heterogeneity (p &lt; 0.10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 (0.47-1.21) 18.7% vs. 19.4%</td>
<td>39</td>
<td>0.16</td>
</tr>
<tr>
<td>0.75 (0.46-1.24)</td>
<td>0.0</td>
<td>0.90</td>
</tr>
<tr>
<td>-0.99 (-1.37 to -0.61)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>-0.01 (-0.31 to 0.29)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>0.35 (0.13 – 0.93)</td>
<td>0.0</td>
<td>0.82</td>
</tr>
<tr>
<td>0.61 (0.28- 1.34)</td>
<td>42</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Bui 8
- No episodes of bacteremia attributed to probiotic use were encountered in the trials that provided relevant information
- 3 of 5 studies had reports of patients (up to 93%) receiving systemic antibiotics at study entry

**Author’s Conclusion**

Probiotics showed an 8.4% decrease in the incidence of VAP and there was no difference in the causative organism in both groups. Though the probiotic group showed decreased ICU LOS and lower colonization with *P. aeruginosa*, this was not statistically significant. There was no difference in any other secondary outcomes.

**Strengths**

- Inclusion of only randomized control trials
- Specific population inclusion criteria (mechanical ventilation > 48 hours and referred to VAP)
- Concurrent antibiotic use in > 90% of the patients enrolled

**Limitations**

- Publication bias was not assessed
- Use of both probiotic and synbiotics
- Variability in strains and dosing of probiotics
- Varied diagnostic criteria for VAP
- Did not discuss severity of illness

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**Objective**

To evaluate the efficacy and safety of probiotics for the prevention of VAP in adult patients undergoing mechanical ventilation.

**Study Design**

- Studies published between 2008-2011
- Sample size ranged from 44 to 259 patients
- 7 randomized controlled trials included

**Inclusion Criteria**

- Adult inpatients undergoing mechanical ventilation
- Probiotics compared to control
- VAP specifically defined
- Data available on the incidence of VAP

**Exclusion Criteria**

- Studies that did not specifically refer to VAP
- Excluded based on titles and abstracts
- Duplicate studies excluded

**Outcomes**

- Primary: Incidence of VAP
- Secondary: ICU and hospital mortality, UTI, CRBSI, diarrhea, ICU and hospital length of stay, and duration of mechanical ventilation

**Methods**

- 7 randomized controlled trials included with a total of 1142 patients
- 5 of 7 studies tested probiotics *Lactobacillus plantarum 299*, *Lactobacillus rhamnosus GG*, and *Lactobacillus casei rhamnosus*
- 2 of 7 studies tested Synbiotic 2000FORTE
- Probiotics were administered via the stomach or mouth (1- solely mouth, 1- mouth and stomach, most- solely stomach)
- Probiotics were administered BID in the majority of the studies (2- administered once daily)

**Statistical Analysis**

- Analyzed as odds ratio with 95% confidence intervals
- *I*² statistic for heterogeneity
- Sensitivity analyses
- Begg funnel plots for publication bias
Results

- Baseline characteristics: patients >16 years of age requiring mechanical ventilation > 24 hours
- Primary outcome: VAP incidence (17% vs. 19.8%)

<table>
<thead>
<tr>
<th>p-value</th>
<th>$I^2$,%</th>
<th>p-value for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35</td>
<td>36.5</td>
<td>0.15</td>
</tr>
</tbody>
</table>

- Further exclusion of any single study did not materially alter the overall combined OR
- Secondary outcomes:

<table>
<thead>
<tr>
<th>p-value</th>
<th>$I^2$,%</th>
<th>p-value for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU mortality (n=727)</td>
<td>0.56</td>
<td>0.0</td>
</tr>
<tr>
<td>Hospital mortality (n=513)</td>
<td>0.10</td>
<td>0.0</td>
</tr>
<tr>
<td>UTI (n=424)</td>
<td>0.30</td>
<td>70.0</td>
</tr>
<tr>
<td>CRBSI (n=424)</td>
<td>0.33</td>
<td>70.6</td>
</tr>
<tr>
<td>Diarrhea (n=426)</td>
<td>0.98</td>
<td>0.0</td>
</tr>
<tr>
<td>ICU LOS (n=305)</td>
<td>0.80</td>
<td>0.0</td>
</tr>
<tr>
<td>Hospital LOS (n=305)</td>
<td>0.66</td>
<td>0.0</td>
</tr>
<tr>
<td>Duration of MV (n=138)</td>
<td>0.93</td>
<td>--</td>
</tr>
</tbody>
</table>

Author's Conclusion

Probiotics show no beneficial effect in patients who are mechanically ventilated and should not be recommended for routine clinical application. Probiotics also failed to show any beneficial effects in the secondary outcomes. Results of this meta-analysis should be interpreted with caution and future studies should focus on safety.

Strengths

- Inclusion of only randomized control trials
- Specific population inclusion criteria
- Exclusion of any single study from statistical analysis (to increase robustness)

Limitations

- No specific exclusion criteria
- No quantitative description of power needed
- Did not discuss antibiotic use
- Publication bias assessed, but limited interpretability due to low power
- Use of both probiotic and synbiotics
- Variability in strains and dosing of probiotics
- Varied diagnostic criteria for VAP
- Did not discuss severity of illness

15. Summary of the meta-analysis:

a. Contradiction between the two meta-analysis
b. 689 versus 1142 cases
c. Gu et al included four recent RCTS that clearly stated enrollment of patients undergoing mechanical ventilation and specifically required VAP
d. Gu et al. excluded two RCTS from the previous analysis done by Siempos et al.
   i. The studies provided data on pneumonia and respiratory tract infections, but did not meet the criteria for VAP
e. Both studies had the use of both probiotics and synbiotics
f. In the Gu et al. trial, total incidence of VAP was 18.4% (210/1142)
g. In the Siempos et al. trial, total incidence of VAP was 23.5% (162/389)
<table>
<thead>
<tr>
<th>Objective</th>
<th>To investigate the effect of oral administration of a probiotic, namely <em>Lactobacillus</em>, on gastric and respiratory tract colonization and infection with <em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrollment</td>
<td>208 patients in a French ICU between March 2003 and October 2004</td>
</tr>
<tr>
<td>Study Design</td>
<td>Prospective, double-blinded, randomized, placebo controlled trial</td>
</tr>
</tbody>
</table>
| Inclusion Criteria | • ≥ 18 years of age  
• Hospital stay > 48 hours  
• Naso-gastric tube feeding |
| Exclusion Criteria | • Immunosuppression  
• Absolute neutrophil count < 500/mm$^3$  
• Gastrointestinal bleeding  
• Contraindications to enteral feeding  
• Isolation of *P. aeruginosa* from gastric aspirates or respiratory tract specimens during the first four days after admission |
| Outcomes | • Primary: time of first *P. aeruginosa* acquisition  
• Secondary: times of *P. aeruginosa* respiratory tract infection or colonization, *P. aeruginosa* gastric colonization, and number of patients with persistent gastric colonization with *L. casei rhamnosus* |
| Methods | • Equal randomization was performed through a computer-generated random allocation  
• Intervention:  
  • Patients received placebo or probiotic (10$^9$ colony forming units of *Lactobacillus casei rhamnosus*) twice daily through NG tube or by mouth from the third day after admission to discharge or death  
  • The presence of *P. aeruginosa* was determined:  
    • With NG tube; collected at admission, once weekly (as long as NG tube was present) and at discharge  
    • With enteral nutrition; gastric aspirates were collected before feeding bottle change (12 hours after probiotic administration)  
    • When no gastric residual was present, 10 mL saline was injected into the tube and aspirated |
| Statistical Analysis | • Sample size target: 200 patients in each arm  
• Two-tailed Fisher exact test or X$^2$ test for qualitative variables  
• Student’s t-test or Mann-Whitney test for quantitative variables  
• Statistical significance was established as *p*-value < 0.05  
• Cox proportional hazards model for independent risk factors of *P. aeruginosa* acquisition |
Results

- 236 patients were randomized
- Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n= 106)</th>
<th>Probiotic (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57</td>
<td>60</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>81: 25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65: 37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAPS II (mean ± SD)</td>
<td>44.2 ± 15.3</td>
<td>44.6 ± 16.0</td>
</tr>
<tr>
<td>Hospital LOS (days)</td>
<td>13.5</td>
<td>14</td>
</tr>
<tr>
<td>Gastric tube (days)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Antibiotic treatment</td>
<td>105</td>
<td>101</td>
</tr>
<tr>
<td>Antipseudomonas drugs</td>
<td>43</td>
<td>55</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p-value < 0.05

- Outcomes:

<table>
<thead>
<tr>
<th></th>
<th>Gastric aspirate Placebo</th>
<th>Probiotic</th>
<th>Respiratory Placebo</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td># of patients acquiring P. aeruginosa</td>
<td>6</td>
<td>3</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Median time before acquisition (days)</td>
<td>30</td>
<td>16</td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>% NA at day 21</td>
<td>97.4</td>
<td>94.7</td>
<td>85.3</td>
<td>98.5</td>
</tr>
<tr>
<td>% NA at day 42</td>
<td>84.4</td>
<td>94.7</td>
<td>70.9</td>
<td>93.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>p-value < 0.05; NA= not acquired

- Overall, 17 versus 6 patients had P. aeruginosa isolates in the body (p= 0.02)
- VAP incidence: 7.5% versus 2.9% (p > 0.05)
- P. aeruginosa was responsible for VAP in 8 patients in the placebo group and 3 in the probiotic group (p > 0.05)
- L. casei rhamnosus was detected in the gastric aspirate of 52/102 patients in the probiotic group
- No patient contracted Lactobacillus associated infection during the study
- Based on Cox regression analysis, absence of probiotic and weight were identified as independent factors associated with increased risk for P. aeruginosa respiratory infection or colonization

Author’s Conclusion

Oral and enteral administration of probiotic in critically-ill patients delayed the time to acquire P. aeruginosa colonization in the respiratory tract; however, this finding did not translate to a statistically significant difference in the incidence of VAP among the two groups. Overall this study suggests a delayed respiratory tract colonization and infection, but showed no beneficial effect on the occurrence of P. aeruginosa VAP incidence.

Strengths

- Study design
- VAP clearly defined
- Accounted for antibiotic use

Limitations

- Patient population limited to single ICU
- Did not meet the planned subject target

<table>
<thead>
<tr>
<th>Objective</th>
<th>To assess the effects of prophylactic probiotic administration in patients ventilated for ≥ 2 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrollment</td>
<td>167 patients in a French ICU between February 2006 and March 2008</td>
</tr>
<tr>
<td>Study Design</td>
<td>Double-blinded, randomized, placebo controlled trial</td>
</tr>
<tr>
<td>Inclusion Criteria</td>
<td></td>
</tr>
</tbody>
</table>
- ≥ 18 years of age  
- Mechanically ventilated ≥ 48 hours |
| Exclusion Criteria |  
- < 18 years of age  
- Predicted mechanical ventilation < 48 hours  
- Pregnancy  
- Immunosuppression (AIDS and cytostatic chemotherapy ≤ 3 months before admission)  
- Short bowel disease  
- Inclusion in another trial |
| Outcomes |  
- Primary: 28-day mortality  
- Secondary: 90-day mortality, reversal of organ failure, occurrence of ICU-acquired infections and colonization by day 28, and ICU LOS |
| Methods |  
- Eligible patients were randomly assigned to a 1:1 ratio of probiotics or placebo  
- Probiotic administered was Ergyphilus® capsules  
  - Multi-species probiotics containing $2 \times 10^{10}$ colony forming units of mainly *Lactobacillus rhamnosus* GG, but also *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*  
  - Intervention:  
    - Patients received 5 capsules of Ergyphilus® or placebo thru enteral feeding tube once daily  
    - After weaning from the ventilator, treatment was given for two additional days in the case of successful extubation or continued in the case of extubation failure  
    - All patients received enteral nutrition within 24 hours of admission starting at 10 kcal/kg (and then increased to 30-35 kcal/kg) |
| Statistical Analysis |  
- Sample size target: 740 patients  
- 90% power at $\alpha$ risk of 0.05  
- Blinded interim analysis planned after randomization of 200 patients  
- Kolmogorov-Smirnov test was used to assess whether continuous data were normally distributed  
- Student’s t-test or Mann-Whitney test for quantitative variables  
- Kaplan-Meier curves with log-rank tests  
- Logistic regression models used for pre-specified subgroup analyses (severe sepsis and non-severe sepsis patients)  
- All analyses were done on the basis of the intention to treat principle  
- A two-sided $p$-value less than 0.05 was deemed statistically significant |
Results

- 167 patients were eligible for randomization; patients were most often excluded due to metabolic disorders or suicide attempts

Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=80)</th>
<th>Probiotic (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years ± SD)</td>
<td>61.8 ± 15.5</td>
<td>59.1 ± 15.9</td>
</tr>
<tr>
<td>Sex (male; n)</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>SAPS II (mean ± SD)</td>
<td>60.5 ± 19.6</td>
<td>58.6 ± 17.3</td>
</tr>
<tr>
<td>SOFA score at admission</td>
<td>9.7 ± 4.8</td>
<td>9.0 ± 4.6</td>
</tr>
<tr>
<td>Antibiotic treatment</td>
<td>31</td>
<td>27</td>
</tr>
</tbody>
</table>

No difference in baseline characteristics

Outcomes:

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=80)</th>
<th>Probiotic (n=87)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28-day mortality</td>
<td>23.7%</td>
<td>24.5%</td>
<td>0.80</td>
</tr>
<tr>
<td>Secondary:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-day mortality</td>
<td>30%</td>
<td>31%</td>
<td>0.90</td>
</tr>
<tr>
<td>ICU LOS</td>
<td>20.2 ± 20.8</td>
<td>18.7 ± 12.4</td>
<td>--</td>
</tr>
<tr>
<td>Overall ICU-acquired infections</td>
<td>37.5%</td>
<td>34.4%</td>
<td>0.68</td>
</tr>
<tr>
<td>VAP</td>
<td>18.7%</td>
<td>26.4%</td>
<td>NS</td>
</tr>
<tr>
<td>CRBSI</td>
<td>6.78%</td>
<td>1.84%</td>
<td>0.005</td>
</tr>
</tbody>
</table>

- No effect on hospital LOS, organ failure resolution and multi-drug resistant bacteria nasal/rectal colonization
- Probiotic or placebo treatment was administered within 2.42 ± 1.84 days after ICU admission
- Unplanned interim analysis was performed after inclusion of first 167 patients to verify safety after the Besselink study was published
  - Committee recommended to stop the study since it was re-calculated that greater than 4000 patients would be necessary to establish treatment effect
- Subgroup analyses:
  - **Severe sepsis:**
    - 28-day mortality: OR= 0.38 (95% CI 0.16-0.93, p-value = 0.035)
    - 90-day mortality: probiotic = 25% vs. placebo=41%
      HR= 0.52 (95% CI 0.26-1.04)
  - **Non-severe sepsis:**
    - 90-day mortality: HR = 3.40 (95% CI 1.18-7.64, p-value= 0.02)
    - Patients in the probiotic group had higher mortality at 28 and 90 days versus the placebo group
    - There were no side effects observed during the study period
    - No bacteremia due to lactic acid bacteria and no bowel ischemia observed

Other Literature

- Besselink MG et al. study29,30,31
  - No bowel ischemia or death
  - Only one patient had acute pancreatitis included in the probiotic group
  - Probiotics strains differed

Author's Conclusion

Probiotic use in the critically-ill who are mechanically ventilated for ≥ 48 hours did not show any statistical benefit in regards to 28 and 90 day mortality and ICU-acquired infections. The prophylactic administration of probiotics cannot be supported in critically ill patients, especially in non-severe sepsis patients, due to lack of an observed protective effect.

Strengths

- Study design
- VAP clearly defined

Limitations

- Prematurely stopped due to the Besselink study
- Patient population limited to single ICU
- Various strains of probiotics were used

### Objective

To determine if the administration of *Lactobacillus rhamnosus* GG in mechanically ventilated patients would reduce the incidence of ventilator-associated pneumonia in a select ICU population and examine the safety of probiotic use.

### Enrollment

146 patients in a university-based hospital between July 2004 to January 2009.

### Study Design

Prospective, double-blinded, randomized, placebo control trial.

### Inclusion Criteria

- ≥ 19 years of age
- 95% likelihood to require mechanical ventilation with an endotracheal tube for at least 72 hours
- Initial intubation during hospitalization
- Approval of the attending physician
- Informed surrogate consent within 24 hours of intubation

### Exclusion Criteria

- Pregnancy
- Immunosuppression
  - Pharmacologic: >10 mg prednisone daily or equivalent for at least 14 days
  - Native: known HIV or AIDS, history of malignancy, multiple organ system failure
- Prosthetic cardiac valve or vascular graft
- Cardiac trauma
- History of rheumatic fever
- Endocarditis
- Congenital cardiac abnormality
- Gastroesophageal or intestinal injury, or foregut surgery during current admission
- Oropharyngeal mucosal injury
- Placement of tracheostomy
- Unable to obtain informed written consent and administer the first dose of the study drug within 24 hours of intubation

### Outcomes

- Primary: microbiologically confirmed VAP incidence based on quantitative BAL culture with at least $10^4$ cfu/mL in patients intubated for 48 hours or longer
- Secondary: mortality, time to occurrence of VAP, duration of mechanical ventilation, ICU or hospital LOS, *Clostridium difficile*-associated diarrhea, other ICU-associated diarrhea, antibiotic consumption and hospital charges

### Methods

- Eligible patients were randomly assigned to a 1:1 ratio of probiotics or placebo using permutation blocks
  - 3 APACHE scores < 18, 18-24, >24
- Intervention:
  - Patients received placebo or probiotic ($2 \times 10^9$ colony forming units of *Lactobacillus rhamnosus* GG) twice daily until extubation, tracheostomy placement or death
  - One probiotic or placebo capsule was given through both the oropharynx and nasogastric tube
  - All patients received routine care (VAP preventive measures as per hospital protocol)
- Quantitative cultures by nonbronchoscopic bronchoalveolar lavage were taken from patients clinically diagnosed with VAP
- All patients with diarrhea had *Clostridium difficile* cytotoxic assay sent; negative assays were repeated twice
- Measurement of flora:
  - Patients had an oral swab, gastric aspirate, and nonbronchoscopic BAL collected before treatment, after 72 hours of study participation, and with clinical diagnosis of VAP

### Statistical Analysis

- Sample size target: 146 patients total
  - To achieve a statistical power of 80% with a two-sided significance level of 0.05
- Student’s $t$-test or Mann-Whitney test was used to compare between group differences for continuous variables
- $X^2$ test for categorical variables
- Modified intention to treat analyses (patients intubated for ≥ 48 hours) were analyzed
- All patients enrolled were analyzed for safety
- Kaplan-Meier analyses were performed
- Wilcoxon Signed Rank test for statistical comparisons
- All $p$-values were two-sided, significance and set at $p < 0.05$
Results
146 patients were randomized; 8 were excluded due to exclusion criteria

Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=73)</th>
<th>Probiotic (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD )</td>
<td>54.6 ± 16.3</td>
<td>52.5 ± 19.3</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>43: 30</td>
<td>43: 30</td>
</tr>
<tr>
<td>APACHE II (mean ± SD)</td>
<td>23.7 ± 8.0</td>
<td>22.7 ± 7.5</td>
</tr>
<tr>
<td>Chest trauma (n)</td>
<td>2⁺</td>
<td>13³</td>
</tr>
</tbody>
</table>

² p-value < 0.05

Primary outcome:

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Probiotic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention to treat analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically diagnosed VAP</td>
<td>33/73 (45.2%)</td>
<td>17/73 (23.3%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Microbiologically confirmed VAP</td>
<td>28/73 (38.4%)</td>
<td>13/73 (17.8%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Modified intention to treat analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically diagnosed VAP</td>
<td>33/70 (47.1%)</td>
<td>17/68 (25%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Microbiologically confirmed VAP</td>
<td>28/70 (40.0%)</td>
<td>13/68 (19.1%)</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

Gram negative pneumonia was significantly higher in the placebo group 22.8% compared to Lactobacillus GG group 8.8%; p= 0.02

Secondary outcomes:

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=73)</th>
<th>Probiotic (n=73)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>21.4%</td>
<td>17.6%</td>
<td>0.42</td>
</tr>
<tr>
<td>C. difficile diarrhea</td>
<td>18.6%</td>
<td>5.8%</td>
<td>0.02</td>
</tr>
<tr>
<td>Days of ICU-associated diarrhea (mean ± SD)</td>
<td>5.9 ± 3.8</td>
<td>4.1 ± 3.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Total antibiotic days prescribed for VAP (mean ± SD)</td>
<td>8.6 ± 10.3</td>
<td>5.6 ± 7.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Total antibiotic days prescribed for C. difficile (mean ± SD)</td>
<td>2.1 ± 4.8</td>
<td>0.5 ± 2.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Durations of mechanical ventilation, ICU and hospital LOS, and hospital charges were not different between the groups

No adverse events due to probiotic administration were encountered

No cases of Lactobacillus bacteremia or pneumonia were seen in the intervention arm

Surveillance culture data:
- At baseline the rates of oral and gastric colonization were not significantly different between groups
- After 72 hours of study participation:
  - Oral colonization rates were higher in placebo (70%) vs. probiotic (38.2%); p <0.001
  - Gastric colonization rates were higher in placebo (45.7%) vs. probiotic (32.3%); p= 0.03

Author's Conclusion
This trial suggests that Lactobacillus rhamnosus GG is safe and efficacious in preventing VAP in a select, high-risk ICU population.

Strengths
- Study design
- VAP clearly defined
- Routine VAP preventive measures were performed
- Used several routes of administration

Limitations
- Single-centered
- Strict inclusion and exclusion criteria makes it difficult to generalize findings to and ICU population
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Intervention</th>
<th>VAP incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forestier et al.</td>
<td>Prospective, double-blinded,</td>
<td>10⁷ CFU <em>Lactobacillus casei</em> rhamnosus twice daily</td>
<td>Probiotic- 2.9%</td>
</tr>
<tr>
<td></td>
<td>randomized, placebo control trial</td>
<td>via NG tube</td>
<td>Placebo- 7.5%</td>
</tr>
<tr>
<td>Barraud et al.</td>
<td>Double-blinded, randomized,</td>
<td>5 <em>Ergyphilus</em> 2 x 10¹⁰ CFU enteral feedings</td>
<td>Probiotic- 26.4%</td>
</tr>
<tr>
<td></td>
<td>placebo control trial</td>
<td></td>
<td>Placebo- 18.7%</td>
</tr>
<tr>
<td>Morrow et al.</td>
<td>Prospective, double-blinded,</td>
<td><em>Lactobacillus rhamnosus</em> GG 2 x 10⁹ CFU twice</td>
<td>Probiotic- 9.1%</td>
</tr>
<tr>
<td></td>
<td>randomized, placebo control trial</td>
<td>daily via NG tube and oropharynx</td>
<td>Placebo- 40.0%</td>
</tr>
</tbody>
</table>

17. Safety of Probiotics

a. Commercially marketed in the United States as a dietary supplement
   i. Food and Drug Administration does not require the same rigorous efficacy and safety regulations compared to conventional pharmaceutical products
b. No safety issues have been identified in the investigations of using probiotics for VAP prevention
c. *Saccharomyces* fungemia is the most severe complication secondary to probiotic administration¹¹,¹⁶,¹⁷
   i. Predisposing factors- immunosuppression, central venous catheters, and digestive tract diseases
   ii. Entry of organisms from healthcare workers’ contaminated hands to patients’ bloodstream during administration
d. *Lactobacillus* bacteremia and endocarditis have lead to fatal comorbidities¹²,¹⁵
   i. Predisposing factors- immunosuppression, prior prolonged hospitalization, and prior surgical intervention
   ii. However, *lactobacillus* has been used in transplant populations with no documented adverse effects, and in HIV- positive patients receiving antiretroviral therapy with no complications
18. Conclusions

a. Forestier et al.
   i. Administration of probiotic in critically ill patients delayed the time to acquire *P. aeruginosa* colonization in the respiratory tract
      1. However this not translate to a statistically significant decrease in VAP incidence
   ii. Probiotics do play a role in altering gastrointestinal and gut flora
      1. Unsure if this is this directly related to the development of VAP
   iii. Study shows a trend towards decrease in VAP incidence but is underpowered

b. Barraud et al.
   i. Probiotic prophylaxis did not reduce VAP incidence and was associated with an increase risk of mortality in patients with predicted severe acute pancreatitis
   ii. Use cannot be supported in critically ill patients, especially in non-severe sepsis, due to lack of an observed protective effect

c. Morrow et al.
   i. Specifically *Lactobacillus rhamnosus* GG is shown to prevent and decrease the incidence of VAP
   ii. Use cannot be generalized to an ICU population due to strict inclusion and exclusion criteria

d. Not all probiotics are created equal where safety and efficacy profiles may depend of strain type

e. Inconclusive evidence due to variability across studies amongst probiotics strain, dosing, duration, route of administration, and diagnostic criteria for VAP

f. Future direction
   i. Larger, multicenter clinical trials with more liberal inclusion criteria are needed
      1. Extrapolation of that data to larger at-risk population
   ii. More focus on the safety of probiotics
   iii. More rigorous studies with other scientists to understand the mechanism of probiotic’s effect
   iv. Optimal probiotic therapy for ventilator associated pneumonia remains unknown
References:


Appendices:

**Appendix A** - Risk factors for MDR pathogens causing VAP ²

<table>
<thead>
<tr>
<th>factor</th>
<th>Study Population</th>
<th>Total number enrolled</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial therapy in preceding 90 days</td>
<td>General patients, &gt;16 years, requiring mechanical ventilation (MV) &gt;2 days</td>
<td>300</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>Current hospitalization of 5 days or more</td>
<td>Medical-surgical patients, &gt;18 years, requiring MV &gt; 2 days</td>
<td>236</td>
<td>Lactobacillus casei rhamnosus 10⁵</td>
</tr>
<tr>
<td>High frequency of antibiotic resistance in the community or in the specific hospital unit</td>
<td>General patients, &gt;18 years requiring MV &gt; 24 hours</td>
<td>50</td>
<td>Lactobacillus planatrum 299, 10¹⁰ CFU</td>
</tr>
<tr>
<td>Presence of risks factors for healthcare-associated pneumonia:</td>
<td>Surgical/multiple injured patients, &gt;18 years requiring MV and at least a 4 day ICU stay</td>
<td>132</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>-Hospitalization for 2 days or more in the preceding 90 days</td>
<td>Surgical/severe multiple trauma patients, &gt;18 years requiring MV</td>
<td>77</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>-Residence in a nursing home or extended care facility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Home infusion therapy (including antibiotics)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Family member with MDR pathogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive disease and/or therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Appendix B** - CPIS¹

<table>
<thead>
<tr>
<th>CPIS points</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal secretions</td>
<td>Rare</td>
<td>Abundant</td>
<td>Abundant + purulent</td>
</tr>
<tr>
<td>Infiltrate on chest x-ray</td>
<td>None</td>
<td>Diffuse</td>
<td>Localized</td>
</tr>
<tr>
<td>Temperature (°F)</td>
<td>97-100.9</td>
<td>101-102</td>
<td>&lt;97 or &gt;102</td>
</tr>
<tr>
<td>WBC count (1000/mm³)</td>
<td>4-11</td>
<td>&lt;4 or ≥11</td>
<td>&lt;4 or ≥11 + &gt; 500 bands</td>
</tr>
<tr>
<td>PaO₂/FiO₂</td>
<td>With clinical ARDS or with P/F &gt;240</td>
<td>Without clinical ARDS or with P/F &lt;240</td>
<td></td>
</tr>
</tbody>
</table>

**Appendix C** - Siempos et al.²³

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Study design</th>
<th>Study Population</th>
<th>Total number enrolled</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knight et al./2009</td>
<td>Double blind, single centered, randomized control trial</td>
<td>General patients, &gt; 16 years, requiring mechanical ventilation (MV) &gt;2 days</td>
<td>300</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>Forestier et al./2008</td>
<td>Double blind, single centered, randomized control trial</td>
<td>Medical-surgical patients, &gt; 18 years, requiring MV &gt; 2 days</td>
<td>236</td>
<td>Lactobacillus casei rhamnosus 10⁵</td>
</tr>
<tr>
<td>Klarin et al./2008</td>
<td>Open-label, single centered, randomized control trial</td>
<td>General patients, &gt;18 years requiring MV &gt; 24 hours</td>
<td>50</td>
<td>Lactobacillus planatrum 299, 10¹⁰ CFU</td>
</tr>
<tr>
<td>Spindler-Vesel et al./2007</td>
<td>Single centered, randomized control trial</td>
<td>Surgical/multiple injured patients, &gt;18 years requiring MV and at least a 4 day ICU stay</td>
<td>132</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>Kotzampassi et al./2006</td>
<td>Double blind, multi-centered, randomized control trial</td>
<td>Surgical/severe multiple trauma patients, &gt;18 years requiring MV</td>
<td>77</td>
<td>Synbiotic 2000 Forte</td>
</tr>
</tbody>
</table>

**Appendix D** - Gu et al.²⁴

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Study design</th>
<th>Study Population</th>
<th>Total number enrolled</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forestier et al./2008</td>
<td>Double blind, single centered, randomized control trial</td>
<td>Medical-surgical patients, &gt; 18 years requiring MV &gt; 2 days</td>
<td>236</td>
<td>Lactobacillus casei rhamnosus 10⁵</td>
</tr>
<tr>
<td>Klarin et al./2008</td>
<td>Open-label, single centered, randomized control trial</td>
<td>General patients, &gt;18 years requiring MV &gt; 24 hours</td>
<td>50</td>
<td>Lactobacillus planatrum 299, 10¹⁰ CFU</td>
</tr>
<tr>
<td>Knight et al./2009</td>
<td>Double blind, single centered, randomized control trial</td>
<td>General patients, &gt; 16 years, requiring (MV) &gt;2 days</td>
<td>300</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>Giamarellos-Bourboulis et al./2009</td>
<td>Double blind, randomized control trial</td>
<td>Patients with ≥ 2 organ system trauma requiring MV</td>
<td>72</td>
<td>Synbiotic 2000 Forte</td>
</tr>
<tr>
<td>Barraud et al./2010</td>
<td>Prospective, double blind, randomized control trial</td>
<td>&gt; 18 years requiring MV &gt; 2 days</td>
<td>167</td>
<td>5 Ergyphilus (2 x 10¹⁰ CFU)</td>
</tr>
<tr>
<td>Morrow et al./2010</td>
<td>Prospective, double blind, randomized control trial</td>
<td>&gt; 19 years requiring MV &gt; 3 days</td>
<td>138</td>
<td>Lactobacillus rhamnosus</td>
</tr>
<tr>
<td>Oudhuis et al./2011</td>
<td>Randomized control trial</td>
<td>&gt; 18 years requiring MV &gt; 2 days and/or expected ICU stay &gt; 3 days</td>
<td>254</td>
<td>Lactobacillus planarum</td>
</tr>
</tbody>
</table>

**Appendix E**³,²⁵,²⁶

<table>
<thead>
<tr>
<th>Study</th>
<th>VAP definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forestier et al.</td>
<td>≥ 1 positive sample or endotracheal aspirate, ≥ 1 new abnormal and progressive radiographic infiltrate, and ≥ 1 of the following signs: purulent sputum production, fever, pathogenic bacteremia without another source, and bronchoalveolar minilavage with &gt; 5% cells with intracellular bacteria</td>
</tr>
<tr>
<td>Barraud et al.</td>
<td>Persistent infiltrate on chest radiograph and associated with ≥ 1 of the following: purulent tracheal secretions, fever, leukocytosis, positive quantitative culture from bronchoalveolar lavage</td>
</tr>
<tr>
<td>Morrow et al.</td>
<td>New, persistent infiltrate on chest radiographs with ≥ 2 of the following: fever, leukocytosis or leukemia, and/or purulent sputum; confirmed with quantitative cultures obtained by nonbronchoscopic bronchoalveolar lavage</td>
</tr>
</tbody>
</table>