Probiotics in Trauma: Curing or Causing Disease?
NW Annual Trauma Meeting
May 10th 2013

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Medical Director Hospital Nutrition Support
Oregon Health and Sciences University
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Probiotics in Clinical Medicine: Two Schools of Thought!

Probiotics “Microorganisms normally present in the human body that when delivered in adequate amounts, confer a health benefit on the host”

Prebiotics: Substrate for probiotic fermentation

Quakery
- Claims to cure “everything”
- Why so many strains?
- How much is needed?
- Review articles vs original articles
- Inconsistent studies
- Few ITT studies with tangible outcomes
- Marginal statistics
- Growing suspicion of “holoistic” medicine

Therapy
- Differential support based on cultural and educational background
  (yogurt, kurd, kefir, kombucha)
- Aware of the differences in strains etc
- Understand the differences in study design
- Few well designed PRCT with ITT analysis
What are they?

Probiotics: live microorganisms that confer a health benefit on the host when administered in adequate amounts.

Prebiotics: substrate for probiotics

Where found?

Probiotics: found in fermented foods

Prebiotics: found in many unprocessed foods

Probiotics + Prebiotics = Synbiotics

Starting from day one we are exposed to pro and prebiotics.
Gastroenterologist Survey: Probiotics

- Evaluate MD opinions regarding probiotics
- Large metropolitan area in midwest
- Results:
  - Safe for most patients 100%
  - 98% felt probiotics had a role in treating GI disease
  - 93% had patients currently taking probiotics
  - Most common bacteria used
    - Yogurt based, B.infantis (Align®), VSL#3,
  - Most common clinical diagnosis used
    - IBS, AAD, C.difficile
  - Most believed their practice was *not supported by scientific data*

Williams MD J Clin Gastro 2010
Probiotic literature? Science or Quackery

• Professional Literature
  • Few ITT studies available
  • Widely variable, heterogeneous groups, variety of bacteria
  • Meta-analysis not consistent
    – Questions of outcome
  • 2009 analysis of what is published probiotics vs antibiotics in PubMed Search
    – Probiotics > 5000 papers ----- 28% were review articles
    – Antibiotics > 500,000 papers----8% review articles

• Lay literature
  • Recent lead articles
    – NY Times 2012
    – Wall Street Journal 2012
    – Economist 2012
Some say we should be killing our bacterial!!
Where “man meets microbe”
a dynamic interplay

- Concepts are not new
  - Biblical references
  - Metchnikoff early 1900’s
- 300 to 400 sq meter surface area
  - Surface area of a tennis court
- > 3 million genes in the bacterial genome vs 30,000 in the human
  - 100 trillion living bacteria in the human intestine
  - Over 700 species in human colon, many non-culturable
  - Each individual with own bacterial fingerprint
  - Extensive # of microenvironments (skin, R v L hand etc)
- Significant “cross-talk” between bacteria and host
  - One bacteria species can turn on > 100 genes
  - Toll receptors on dendritic cells / macrophages
  - Gut contains complex neuroendocrine system
- Quorum sensing
  - Molecules secreted by bacteria: partially explain bacterial community behavior and activation of virulence genes etc

Metchnikoff 1906
Does Surgery or Peri-op Therapy Alter the Microbiome?

- Inflammatory changes
- Bacterial interrelationships
- Bacterial changes with host stress situations
  - Bacterial use environmental clues
    - pH, temperature, redox potential, osmolality
  - When energy supply is limited genes “switch on” virulence factors
  - Ex: E.coli and Pseudomonas can rapidly become virulent with host stress (epinephrine, cortisol, morphine etc)

Alverdy J, CCM 31:598-607, 2003
Alverdy J Molecular Biol 2008
Babrowski T et al Ann Surg 255;236-393 2012
Nutritional Goals in Clinical Medicine Have Changed From Adjunctive Care to a “Therapeutic” Strategy

• Previous goals
  • Attempt to preserve lean body mass
  • Avoid metabolic complications

• Current Goals: “Therapy not support”
  • Attenuate metabolic response
  • Reverse loss of lean body tissue
  • Prevent oxidant stress
  • Favorably modulate immune response
    – Enteral feeding (GALT)
    – Appropriate macro and micronutrients
      » Glutamine, arginine, omega-3-FA, antioxidants

• Maintaining “normal” commensal flora
  » Prebiotics

• Manipulating flora to host benefit
  » SCFA, anti-inflammatory changes, decrease sepsis
Probiotics can *prevent, mitigate* and *treat* many of the current health crisis facing the western world

- **Cancer**
  - Multiple mechanisms
- **Heart disease**
  - Metabolic syndrome
  - Atherosclerosis
- **Depression**
- **Hepatic diseases**
  - NASH
- **Infectious disease**
- **Diarrheal diseases**
  - AAD
  - Bacterial
  - C.diff
  - Viral
- **Inflammatory diseases**
  - IBD
  - Allergy
  - Asthma
- **Autoimmune diseases**
- **Aging**
- **Obesity**
- **Renal disease**
- **Critical Care / Surgery**
  - Trauma
  - General surgery
  - Pancreatitis +/-
  - Transplantation
  - Sepsis
  - VAP prevention
  - AAD / C.difficile
Mechanisms:

1. Enhancement of the epithelial barrier

   - Mucins and defensins

2. Increased adhesion to intestinal mucosa

3. Inhibition of pathogen adhesion

4. Competitive exclusion of pathogenic microorganisms

5. Production of anti-microorganism substances

   - Example: bacteriocins

6. Modulation of the immune system

   - IL-10
   - TGFβ

   - Immature DC
   - Macrophage
   - Treg
   - Th1
   - Th2
   - Th17

Mechanisms:

Colonization Resistance
Antimicrobial Factors

**L. reuteri** inhibits **H. pylori**

**L. reuteri** inhibits **Staph aureus**

**Mechanisms**
- Competitive inhibition
- Physical barrier (mucous)
- ↓ Adherence, attachment
- Produce bacteriocins, defensins, trefoil
- Bind pathogens
- ↓ pH reduces growth
- Interferes quorum sensing
  ↓ Virulence expression
- Breaks up biofilms

**Bacteria**
- *Escherichia coli* (pathogenic)
- *Salmonella typhimurium*
- *Shigella* spp.
- *Campylobacter jejuni*
- *Streptococcus mutans*
- *Bacillus subtilis*
- *Clostridium perfringens*
- *Helicobacter pylori*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Pseudomonas fluorescens*

**Fungi**
- *Candida albicans*
- *Aspergillus flavus*
Protecting the mucosal lining:

“Soluble factors for *Lactobacillus rhamnosus GG* activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells”

- 70% of energy for colonocyte derived from luminal butyrate
- Cell culture model
- DNA microarray methods, real-time PCR and electrophorethic mobility shifts studied
- Studies confirm:
  - L. GG modulates signaling pathways
  - Activates via MAP kinase
  - L.GG protects mucosa from oxidant stress via expressing HSP

Tao K, Drabik K, Waypa T
Am J Physiol Cell Physiol 290;1018-1030,2006
Mechanisms: Enhancing mucosal blood flow

Mechanisms: stimulation the immune system in the small intestine of healthy subjects

Before *L. reuteri* intake

- Resting CD4+ T-helper cells

After *L. reuteri* intake

- Activated CD4+ T-helper cells

Clinical Equivalent

Probiotics prior to immunization seasonal flu vaccine:
- Enhances anti-body response
  - Specific IgG, IgG1, IgG3
- No change in inflammation


*Valeur et al., Appl Environ Microbiol 70 1176-1181 (2004)*
Probiotics altering nutrient efficiency and utilization

- **Comparative studies in GF animals**
  - GF require 30% more calories to maintain weight
  - GF require water soluble vitamins to maintain life
  - Obesity studies showing changing microbial patterns alters total body fat stores, nutrient utilization

- **CHO**
  - Primary fuel for colonocyte (SCFA)
  - SCFA required for maximal neutrophil function
  - Multiple metabolic functions, energy, membrane stabilize etc
  - 10% of total calories from fermentable substrate

- **Protein**
  - Estimates up to 20% of lysine, leucine, threonine in *non-ruminants* from gut bacteria
  - D-serine

- **Lipid**
  - In starvation/fasting models liver does not produce ketones for optimal energy utilization – gluconeogenesis from protein stores
The Gut: The Forgotten Organ of Uremia

- Ruminant animals i.e. sheep and cows can survive weeks to months following bilateral nephrectomy
  - Mechanism: utilizing urea and cellulose to make amino acids

- Dairy cows live for six generations, calve, and produce normal milk on a protein-free die

- Human experiments giving daily “ruminant bacteria” to 10 uremic patients decreased azotemia and HTN which increased again after stopping therapy

Schepers Eva et al Blood Purification 2010
Freidman E. Current Med Trends 2009
Poesen R Seminars Dialysis 2013
SCFA = Fermentation end product of some probiotics (from prebiotics)

- Energy source;
  - Colonic mucosa;
    - Stimulates cell proliferation, Promotes sodium and water absorption
  - Cardiac, skeletal Muscle, brain
    - Acetate;
  - Propionate; gluceneogenesis

- Regulation of gene expression for ICAM-1 and E-Selectin on endothelial cells

- Decrease COX-2 expression
  - (butyrate and propionate)

- Prevention of neoplastic transformation
  - Inhibits histone deactylase by DNA hypermethylation to promote differentiation in cancer cell lines

- Enhances Leptin secretion

- Inhibition of pathogen overgrowth in gut lumen, pH control

- ROS scavenger
  - Pyruvate is anti-inflammatory and decrease NFKB expression

- Activation of polymorphonuclear cells
  - Both local and systemic
  - G-protein receptors on circulating PMN’s

Thangaraju M et al J GI Surg 2008
SCFAs, Fiber Fermentation and Butyrate Receptors

- Trophic effect, colonocyte fuel
- Anti-inflammatory
- Enhance WBCs, macrophage
- ↓Adhesion molecules
- ↓microvascular thrombosis

Thangaraju M et al J GI Surg 2008
Ganapathy V 2011
Changes in fecal bacterial products in trauma ICU are predictive of outcome!

- Gut represents most diverse and fragile microenvironment and ecosystem in the body
  - Dramatic alteration by critical illness and broad spectrum antibiotics
- Population in ICU > 48h (N= 491 samples, 138 pts)
  - Acids measured
    - Acetate, lactate, succinate, formate
    - Cytoprotective SFA; propionate, butyrate
- ↑ in pH predicts mortality
  - Loss of anaerobic bacteria

Osuka A, Shimizu K et al. Critical Care 2012

- Probiotics limit mucosal barrier dysfunction and regulate the balance of CD4+ effector lymphocytes

- **Probiotics:**
  - Reduced ICU length of stay 10.7 to 6.8 days
  - Reduced VAP 68% to 44%
  - Reduced mortality 19 to 11%

- Small sample sizes makes it unpowered to show significance of outcome parameters
Conclusions:

Use of probiotics in trauma patients is;

- Associated with reduction in incidence of nosocomial infections
- Reduction in VAP
- Reduction in length of stay

Caution with conclusions as;

- Significant heterogeneity of study design
Areas of Critical Care Where Probiotics Have Reported Benefit

**Treatment:**
- Trauma
- Pancreatitis +/-
- Transplantation
- Sepsis
- NASH

**Prevention:**
- VAP
- Antibiotic associated diarrhea
  - C.duificile
- VRE colonization
Ventilator Associated Pneumonia

- One of most frequently occurring nosocomial infections in the ICU
- Current strategy not working
  - Antibiotics – increases resistant flora
  - Ventilator adjustments – variable success
  - Prokinetic agents – no influence
  - Medications – no influence
  - Surfactants – no influence
  - Mouth wash – variable
  - Etc etc etc
“Oral probiotics and prevention of *P. aeruginosa* infections: a randomized, double-blind, placebo-controlled pilot study in intensive care unit patients”

• **Hypothesis**: oral application of probiotics will prevent the secondary colonization with pathogens

• **PRDBPC trial**
  - Inclusion criteria
    - patients in ICU > 48 hours
  - 807 eligible: 106 placebo vs 102 probiotic completed
  - $10^9$ L. casei BID started day 3 until discharge
  - Monitored gastric and oral bacteria cultures

• **Results**:
  - Delayed colonization of *P. aeruginosa* in respiratory tract

Forestier C Critical Care 2008,12:1-10
Use of Probiotics to Prevent Ventilator Associated Pneumonia

- *Lactobacillus GG* vs placebo (DBPCT)
  - (2871 patients screened 146 met criteria)
  - On vent > 72 hours
  - Oral and via feeding tube
  - $1.0 \times 10^{10}$ BID to each site

- Evaluated
  - Oral flora pathogen vs normal flora
  - Gastric flora pathogen vs normal flora
  - Incidence of VAP

- Results
  - Less antibiotics used
  - Less C. difficile 5.8% vs 18.6% (p<.05)
  - Clinical VAP 35% vs 47% (p<.05)
  - Microbiologic VAP 19% vs 40% (p<.05)
  - Mortality 14% vs 24% (NS)

*Morrow S, Kollef M et al 2010 AJRCCM*
Not all Probiotics VAP studies positive:

- N = 259 ICU Mechanical ventilation > 72 h
- Probiotics delivered to GI via tube
  - With soluble fiber
- Results:
  - VAP w/ probiotics 9% vs 13 % in control (NS)
  - Mortality 27% in probiotics vs 33% in control (NS)
- Conclusion:
  - No significant improve in VAP or mortality
- (note: probiotics only given enterally, no oral / pharynx delivery)

Knight DJ Int Care Medicine 2009
Impact of administration of probiotics on VAP: Meta-analysis

- RCT with mechanical ventilation +/- probio
- 5 RCT included
- Results:
  - Probiotics decrease VAP
  - Decrease in Pseudomonas colonization
  - No change in mortality
  - No change in ventilator days

Review of the literature
- Issues to resolve
  - Which bacteria
  - How long
  - What population to deliver to
  - Heterogeneity of literature at this point makes firm conclusion difficult
  - L. rhamnosus seems to be most actively being studies

I Siempos et al Crit Care Med 2010
Bailey JL. Ann Pharmacotherapy 2011
Antibiotic Associated Diarrhea: Preventable or Inevitable?

• Hempel S et al JAMA 2012
• Meta-analysis 82 RCT met criteria for inclusion
• Probiotics strains were poorly documented
• N=11,811 participants (pooled data)
• Conclusion:
  • Probiotics confer significant decrease in AAD (p<.001)
  • # needed to treat N=13
Rising Incidence of C. difficile

- Incidence of C. difficile by year

Pathogenesis of CDAD

Antibiotic therapy

Alteration in colonic microflora

*C. difficile* exposure and colonization

Release of toxin A and Toxin B

Colonic mucosal injury and inflammation

Badger, VO et al JPEN 2012*
Emergence of B1/NAP1 Strain

- Produces 16-23 times C. diff. toxins A and B in vitro,
- represented 50% of isolated strains between 2001-2003
  - Produces a 3rd binary toxin
- Increased risk of relapse
- Less responsive to standard therapies

Major Genes in the Pathogenicity Locus (PaLoc) of Clostridium difficile and Relation to the Genes for Binary Toxin

- McDonald NEJM 2005
Use of probiotic preparations to prevent C. difficile Associated Diarrhea

<table>
<thead>
<tr>
<th>RDBPCT N=135</th>
<th>Meta-analysis 28 studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 64 all taking antibiotics</td>
<td>N=3818 patients</td>
</tr>
<tr>
<td>100 gm BID L. casei as drink</td>
<td>“Moderate quality” of evidence probiotics as prophylaxis</td>
</tr>
</tbody>
</table>

**Results:**

- AAD: 7/57 (12%) vs 19/56 (34%)
- 21% relative risk reduction, NNT 5
- C.diff 0/57 vs 9/53 (17%)

- decreases incidence of CDAD by 66%
- No adverse influence by receiving probiotics


Johnston BC Ann Internal Medicine 2012
The ultimate probiotic: Is stool from a “good friend” or family member the answer for refractory C. difficile diarrhea

- RTC 39 patients with proven refractory C. difficile
- 16 got Donor feces / 13 received QID vancomycin
- Results:
  - **Feces group**
    - 13/16 resolved with single infusion
    - 2/3 resolved with second infusion
  - **Vancomycin group**
    - 4/13 resolved

Nood EV NEJM 2013

Nood EV NEJM 2013

Hamilton MJ et al
Frozen “fecal” prep for C.diff
43 consecutive, recurrent CDI
95% success
Am J Gastroenterology 2012
“Probiotic treatment of VRE: Randomized Controlled Trial.”

- PRPCBT 27 VRE positive patients
- Yogurt (containing live Lactobacillus GG vs Pasteurized yogurt)
- 100 gm daily x 4 weeks
- Primary outcome measure: clearance of VRE
- Results:
  - L.GG group: 11/11 cleared VRE at 4 weeks, 3/11 reconverted + at 4 weeks
  - Control: 1/12 cleared
    » Allowed to crossover at 4 weeks 8/11 crossed over
    » 8/8 of the crossover group cleared in 4 weeks

PRPCBT = Prospective Randomized Placebo Control Blinded Trial
# Pre and Probiotics in the Surgery and ICU Setting

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Population</th>
<th>Design</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Olah</td>
<td>2002</td>
<td>N = 45 pancreatitis</td>
<td>Oat fiber +/- L.Plantarum</td>
<td>Dec infection 4.5 vs 30%</td>
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<td>Olah</td>
<td>2007</td>
<td>Pancreatitis</td>
<td>Oat fiber, multiple probiotics</td>
<td>Decrease infections</td>
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<td>Rayes</td>
<td>2002</td>
<td>N = 60 Abdominal surgery</td>
<td>Oat fiber +/- L.plantarum</td>
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<td>Rayes</td>
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<td>N = 66 Hepatic transplant</td>
<td>Fibers +/- 4 strains probiotics</td>
<td>Dec infection 3 vs 48%</td>
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<tr>
<td>Katumpasi</td>
<td>2007</td>
<td>N=65 Vent, multiple trauma</td>
<td>Synbiotics</td>
<td>Dec infection, SIRS, Sepsis, mortality</td>
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<td>Alberda</td>
<td>2007</td>
<td>N=28 ICU</td>
<td>Probiotics VSL # 3</td>
<td>Enhance immune func</td>
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<td>Springer-vessel</td>
<td>2007</td>
<td>N=113 Trauma</td>
<td>4 groups, Synbiotics</td>
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<td>Chunmao</td>
<td>2007</td>
<td>N = 45 Post op GI cancer</td>
<td>Syn / pre/ TPN</td>
<td>Dec infection 47 v 20 v 7 %</td>
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<td>Author</td>
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<td>GUT 2002</td>
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<td>PRCT</td>
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<td>PRCT</td>
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<td>Ann Surg 2007</td>
<td>Whipples</td>
<td>PRCT</td>
<td>Reduce infection and complications</td>
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<td>Kanazawa H</td>
<td>Langenbecks Arch Surg 2005</td>
<td>Hepatobiliary Cancer</td>
<td>PRCT</td>
<td>Decrease infection</td>
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<td>Sugawara G</td>
<td>Ann Surg 2006</td>
<td>Biliary Cancer</td>
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<td>Besselink MGH</td>
<td>Lancet 2008</td>
<td>Pancreatitis</td>
<td>PRCT</td>
<td>Increase death + probiotics</td>
</tr>
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</table>

**Kinross JM et al JPEN 2013**

A Meta-Analysis of Probiotic and Synbiotic Use in Elective Surgery: Does Nutrition Modulation of the Gut Microbiome Improve Clinical Outcome?

**Conclusion:** “Probiotic and synbiotic nutrition strategies reduce the incidence of postoperative sepsis in elective general surgery…..”
Other areas of interest in surgery

• Pouchitis following IAP
  • 3 major studies with VSL#3 reporting benefit
  • 1 major study with no benefit (single organism)

• Necrotizing enterocolitis in neonatal ICU setting
  • Multiple studies > 16
  • 3 large meta-analysis
  • All but few report benefit in prevention, ? Should be SOC

• Patients requiring PN
  • Mostly animal models
  • Rapidly shown to increase inflammatory cytokines in the mucosal epithelial wall (1)

• ICU
  • 23 RCT “appear to reduce infectious complications – VAP, mortality ? (3)

• Obesity

• GI anastomosis

1. Feng Y, Teitelbaum DH Ann NY Acad Sci 2012
2. Gu WJ et al JPEN 2012
3. Petrof EO CCM 2012
Prevention of GI Anastomosis failure

- Animal models (Alverdy’s group)
  - IR increases mortality with Pseudomonas after inoculation
    - Expression of barrier disrupting adhesin PA-IL
  - Bacteria at sight of anastomosis change phenotype and become more aggressive and produce adhesins and enzymes with increase risk of anastomotic disruption
    - Altered by MBP, antibiotic Bowel Prep, ischemia etc

Fink D, et al J Trauma 2011
Stern JR et al J Surg Res 2013
Prebiotics – Probiotics or Synbiotics

Bringing the Science to Practice

Scientific American 2012
Probiotic Beverages

**Chilled dairy**
- Yakult
- Danactive / Actimel
- Stonyfield
- BioQ

**Chilled non-dairy**
- ProViva
- Good belly
- Komboucha
- Bravo Friscus

**Shelf stable**
- Cocobiotic, Dong Quai, Innergy Biotic
Better probiotic delivery systems

- Keep it away from the liquid until ready to use!
  - Micro encapsulation
  - Packaging solutions
    - Bottle closures
    - Drinking straws
<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Active Ingredient</th>
<th>Form</th>
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<tr>
<td>Florastor</td>
<td><em>Saccharomyces boulardii</em> 250 mg</td>
<td>Capsules</td>
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<tr>
<td>Florastor Kids</td>
<td><em>S boulardii</em> 250 mg</td>
<td>Powder</td>
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<tr>
<td>Align</td>
<td><em>Bifidobacterium infantis</em> 35264 (1 × 10⁹ CFU)</td>
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<td>DanActive</td>
<td><em>Lactobacillus casei</em> DN-114 001</td>
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<td>Sustenex</td>
<td><em>Bacillus coagulans</em> GBI-30, 6086 (BC30)</td>
<td>Capsules, chewies, and gummies</td>
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<td>Floranex</td>
<td><em>Lactobacillus acidophilus</em> (2 × 10⁶ CFU)</td>
<td>Capsules</td>
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<td>Lactinex</td>
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<td>Phillips Colon Health</td>
<td><em>Lactobacillus gasseri</em>, <em>Bifidobacterium bifidum</em>, and <em>Bifidobacterium longum</em></td>
<td>Capsules</td>
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CFU, colony-forming units.
**Table 1. Common Probiotic Preparations Available in the United States**

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<tr>
<td>Floranex</td>
<td><em>Lactobacillus acidophilus</em> (2 x 10^6 CFU)</td>
<td>Capsules</td>
</tr>
<tr>
<td>Lactinex</td>
<td><em>L acidophilus</em> and <em>Lactobacillus helveticus</em> (bulgaricus)</td>
<td>Capsules and packets</td>
</tr>
<tr>
<td>Phillips Colon Health</td>
<td><em>Lactobacillus gasseri, Bifidobacterium bifidum,</em> and <em>Bifidobacterium longum</em></td>
<td>Capsules</td>
</tr>
</tbody>
</table>

*CFU, colony-forming units.*

*NOT a true probiotic*
What’s in a label?

• Marcobal et al tested 14 US commercial probiotic products:
  – 93% incorrectly labeled
  – 57% had contaminants
  – 36% did not list strains on the label

• Masco et al tested 58 different products from EU, UK, Asia, Japan, Canada:
  – Only 38% had the dose stated on the label
  – 29% did not contain strains on the label
Not all *lactobacilli* survive in the GI tract

12 dairy products off the shelf in UK stores

8 with the “correct” bacteria

35 strains of mainly *Lactobacillus* and *Bifidobacterium* isolated

Stomach (pH, enzymes)
Duodenum (enzymes)
Ileum (bile)
Colon (competition)

It is all about “Risk vs. Benefit”
Probiotic Safety:
Generally Recognized as Safe (GRAS) USA
Qualified Perception of Safety (QPS) EU

• Can probiotic species transfer resistance genes?
• Lactobacillus bacteremia
  • 180 cases in 30 years
  • 69 cases of endocarditis in 30 years
    – (majority of L. rhamnosus)
• Hepatic Lactobacillus abscess in transplanted liver and immune compromised host reported
• S. Boulardii
  • Recent data showing several outbreaks of S. Cervisiae fungemia when giving S. Boulardii
  • S. boulardii not true probiotic?
• Host risk factors
  • Immunocompromised
    » This is theoretical, clinical data would support use
  • Recent major dental work (theoretical anecdotal reports)
• Caution in severe pancreatitis (Lancet Feb 2008)
  Sanders ME Ann NY Acad Science 2011
  Salminen MK et al Clinical Infectious Disease 2004
Probiotics in Pancreatitis: Randomized Prospective Multicenter Trial

- Multicenter RDBPC Trial 298 patients ITT analysis
- APACHE > 8, Imrie >3 or CRP > 150
- Assigned within 72 hours of symptoms
- Control N=145  Multispecies probiotic N=153
- 2 weeks of therapy
- Endpoints: Inf nec, BSI, pneumonia, urosepsis etc

Results:
- Infectious complications 30% vs 28%
- Mortality 16% probiotic vs 6% in control

Probiotics in Pancreatitis?

• Majority of deaths were from bowel necrosis
  • No bacteremia with probiotic species
  • Necrosis patchy, not just at site of probiotic delivery

• What happened?
  • More organ failure in exp group at start (13% vs 4% in control)
  • Large number of bacteria (>10 billion)
  • Location of delivery D3 – D4
  • Bowel dysmotility “ileus”
  • Insoluble and soluble fiber in formula
  • ? Localized fermentation, acidosis, necrotic bowel, poor randomization ????

• 2011: FDA changed oversight and stated that probiotics as dietary supplements only applies to healthy people and any use of probiotics to prevent, treat or mitigate disease would define the probiotic as a drug *

*Venugopalan V Emerging Infect Dis 2010
Current Problems with “Probiotic”

- Extravagant claims without data
  - Still perceived as “quackery” by many
- ? of good manufacturing practice
  - Quality assurance
    » Additional species and devoid of label common
  - Label vs content
  - Viability of bacterial species
    » Strain variation, SNP changes ?
- Validate biomarkers for assessing function and activity
- Improve the reliability and ease of taxonomic classification of pre and probiotic
  - Culture independent methods
  - Fermentation index
  - FISH (fluorescent in situ hybridization)
  - 16S ribosomal amplification and sequencing techniques
  - Pulse-field gel electrophoresis
  - Amplified fragment-length typing, terminal restriction polymorphism
  - Multi-locus sequence typing
- No specific guidelines currently
  - USA far behind EU in regulation

Sanders ME Ann NY Acad Sci 2011
Probiotics: So many questions, so few answers !!!

- Monostrain vs multistrain?
- Pre, pro or synbiotic?
- Will cell free extracts work?
- Quantity and quality of probiotic needed for desired effect?
  - Most studies “doses” range from $10^7$ to $10^{12}$
  - What dose in Peds?
- How best to assess the activity / viability?
- Probiotic safety?
- Which Probiotics remain viable in GI tract?
- When are probiotics contraindicated?
- Resistant patterns?
- Immunocompromised host?
Are specific strains really needed or is it “one size fits all”? 

• Specific strains have specific effects
  • Ex: L. acidophilus – great for H. pylori issues not for much else

• Ex: Murine model of leukemia
  • Restoring specific strains benefits in maintaining muscle mass and lowering inflammation
    – Followed expression of muscle atrophy markers
      » Atrogin-1, MuFR1, LC3, Cathepsin L
    – Reported:
      » L. reuteri and L. Johnsonii/gasseri benefited
      » L. murinus/animalis no benefit

# Probiotic Protocols

## OHSU Protocol for Synbiotic Use in Hospitalized Adult Patients Receiving Antibiotics

<table>
<thead>
<tr>
<th>Indications</th>
<th>Patients at risk for developing AAD, CDI (broad spectrum antibiotics, ex: fluoroquinones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraindications</td>
<td>Immunosuppressed patients (ex: BMT) (neutrophil count &lt;500)</td>
</tr>
</tbody>
</table>

## Route & Dosage

<table>
<thead>
<tr>
<th>Route</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>4 ounces Nancy’s Yogurt or Kefir  BID 1 pack Benefiber QID</td>
</tr>
<tr>
<td>Feeding Tube</td>
<td>80 ml Nancy’s Kefir + 1 pack Benefiber + 60ml sterile water TID</td>
</tr>
</tbody>
</table>
## OHSU VAP Prevention Protocol for Adults

<table>
<thead>
<tr>
<th>Indications</th>
<th>Ventilated patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraindications</td>
<td>Immunosuppressed patients (neutrophil count &lt;500)</td>
</tr>
</tbody>
</table>

### Route & Dosage

<table>
<thead>
<tr>
<th>Oropharyngeal</th>
<th>Swabbed with Nancy’s Kefir BID (following oral care)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding Tube</td>
<td>80 ml Nancy’s Kefir + 1 pack Benefiber + 60ml sterile water TID</td>
</tr>
</tbody>
</table>

![Nancy's Kefir Logo](image)  
![Nutrition Facts](image)
## Probiotics Protocols

### Legacy Health Probiotic Protocol for Prevention of AAD

<table>
<thead>
<tr>
<th>Indications</th>
<th>Patients at risk for developing AAD (broad spectrum antibiotics, ex: fluoroquinones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Critically ill patients will be assessed by RD for appropriateness</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contraindications</th>
<th>Pancreatitis, Neutropenic precautions, AIDS (T-Cell count &lt;200)</th>
</tr>
</thead>
</table>

### Route & Dosage

<table>
<thead>
<tr>
<th>Oral Feeding</th>
<th>8 ounces Nancy’s Kefir daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding Tube – gastric only</td>
<td>80 ml Nancy’s Plain Yogurt + 200ml water daily</td>
</tr>
<tr>
<td>For patients with dairy intolerance ??</td>
<td>Culturelle LGG 1 pill BID taken 1 hr before or after antibiotics</td>
</tr>
</tbody>
</table>
# Probiotic Protocols

## Portland VAMC Probiotic Protocol for Hospitalized Patients

<table>
<thead>
<tr>
<th>Indications</th>
<th>Patients at risk for developing AAD, CDI (broad spectrum antibiotics, ex: fluoroquinones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraindications</td>
<td>Neutropenic precautions</td>
</tr>
<tr>
<td><strong>Route &amp; Dosage</strong></td>
<td></td>
</tr>
<tr>
<td>Oral Feeding</td>
<td>100 ml container DanActive® BID</td>
</tr>
<tr>
<td>Feeding Tube</td>
<td>100 ml DanActive® + 60 ml water BID</td>
</tr>
<tr>
<td>Product</td>
<td>Type of Bacteria</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>Nancy’s Yogurt</td>
<td><em>L. acidophilus</em>, <em>L. casei</em>, <em>B. bifidum</em>, <em>L. rhamnosus</em>,</td>
</tr>
<tr>
<td>Nancy’s Kefir</td>
<td><em>L. acidophilus</em>, <em>L. casei</em>, <em>B. bifidum</em>, <em>L. rhamnosus</em>, Prebiotic - inulin</td>
</tr>
<tr>
<td>Culturelle LGG</td>
<td><em>L. GG</em></td>
</tr>
<tr>
<td>Danactive</td>
<td><em>L. casei</em></td>
</tr>
<tr>
<td>Stonyfield</td>
<td><em>Bifidus L. Casei</em></td>
</tr>
</tbody>
</table>
General Guidelines for Use of Probiotics

- Critically evaluate and use only when data supports
  - Base choice on molecular typing, metabolic characteristics and interaction in the environment
  - Caution with meta-analysis, heterogeneity is key
- Do not extrapolate from one strain to another
- Identify optimal strain, insoluble fiber and commercially available product
  - ~Probiotic: $10^{9-11}$ viable cells per day ?
  - ~Prebiotic: 20-30 gm/day ?
- Continued intake of probiotic is required to maintain benefits
- Prebiotic are an excellent option to modify flora on long term basis
  - Persistent levels require continuous intake !
Concepts the clinical team need to understand regarding probiotics!

- **NO** single probiotic meets the need in all patients
  - Effects are often strain specific
- **Consider the disease process:** prevention vs treatment
- **Decision should depend upon:**
  - Metabolic insult or expected insult
  - Timing of delivery; pre, post, or both
  - Severity of condition
  - Expected duration of need
  - Tolerance
  - Function of GI tract remaining
  - Strain by strain assessment
- **Base decision on scientific evaluation of the data**
Future Trends: Probiotics in Clinical Medicine

- More data on specific strains of probiotics
- Better acceptance by “public and scientific community”
- New attention to gut / microbe symbiosis
  - Superorganism concept !!
- Probiotics as drug delivery tools genetically engineered
  - “Designer probiotics”
Probiotics as drug delivery tools!

DSS

$Lactococcus lactis$

IL-10

Not treated

Treated

$p = 0.01$

Histologic Score

IL-10 knockout

$Lactococcus lactis$

IL-10

Not treated

Treated

$p = 0.02$

Science 2000; 289:1352-5 (mice)
Clin Gastroenterol Hepatol 2006;4:754-759 (humans)
Ongoing Trials: Probiotics

- **Neurologic disorders**
  - Pain control, ADHD, Tourette syndrome

- **Inflammatory diseases**
  - Aging, IBD, arthritis, asthma, diabetes

- **Use on non-GI surfaces**
  - Burns, tracheostomy sites, skin in ICU, chronic wounds, STSG, Vagina, respiratory tree
  - Breaking up biofilms

- **AIDS prevention**
  - Changing the pH of the vagina alters HIV receptors
  - Gene transfer HIV receptor into probiotics
    » Already done for L. jensenii (Yamamoto HS BMC Micro 2013)

- **Cancer prevention**
  - Multiple mechanisms
    » Dietary procarcinogens by commensal bacteria
    » Histone deacetylase inhibitor

- **Nephrology**
  - Decrease frequency of dialysis required