Pre- and Probiotic Snake-Oil

The pre- and probiotics is an industry, not a division of medical science
Success is measured in dollars, not outcomes; “Ders money in dem der hills!”
The industry offers an alternative to traditional medicine
Probiotics are regulated as “functional foods” by the FDA, a category that includes vitamins
and minerals and other ill-defined “health foods or supplements”
The FDA need only assure the product is safe for human consumption, but it could kill your dog

Probiotics do not require disclaimers from their manufacturers like pharmaceuticals, e.g. Viagra, Humira
Keytruda, Lyrica, Trulicity, Eliques, Otezla, Jamuvia etc.

If you suffer from high blood pressure or Type II diabetes do not take this medication
This drug can cause depression and lead to suicide
If your erection last for more than four hours, call your physician (or the next suitable partner)
If you have any allergies, avoid the use of this medication
If anyone in your family has any of these conditions, avoid use of these pharmaceuticals
If you suffer from insomnia, frequent violent outburst or kick your dog, do not use this medication
If you have a pacemaker, any titanium implants, this product could cause death
Do not use this drug if you are pregnant or plan to be pregnant sometime in the future

Pharmaceutical disclaimer indicate that these medication are only for healthy people: Probiotics are for everyone!

Changes are occurring
The EU now requires probiotics to meet pharmaceutical standards and nearly all probiotics available
in Europe failed to reach these standard!
The Credibility of Probiotics Claims Parallels Trends in News and Science

**News media**
- News channels are a mixture of poorly separated “facts and “opinions”
- Spin-free newspapers are in decline and social media is now a major source of news
- Trump popularized the fake news system and wealthy ideologues bankroll talk shows that support it

**Medical Science**
- Reviews and opinions have become the major source of information
- Consumers obtain medical information from pharmaceutical firms on TV rather than from physicians (Viagra replaced Lucky Strikes)
- “Pay to play” or “parasitic” online journals publish anything if the investigator is willing to pay their price ($4-26K). These journals increased 300% in 15 yrs while qualified reviewers increased < 10%
- ~300 articles published as peer-reviewed are retracted per year

**How do these societal changes affect information on probiotics?**

The majority of studies on probiotics are published in “pay to play” scientific journals and primarily report studies done by manufactures of probiotics and prebiotics

Many Internet and TV adds for probiotics are based on testimonials, not controlled experimental studies.

*Good read: Jeffrey Kluger (TIME) August 2015.*
Quality Control: Meeting the Definition of a Probiotic

The “unwritten” minimum standard is a product that delivers $10^8 - 10^{10}$ viable bacteria, i.e. cfu per ml.

You need 500-1000 ml of yogurt (2-4 cups) to reach the desired $10^8 - 10^{10}$ daily minimum of bacteria to be effective. [Activa claims one billion per container, i.e. $10^{10}$.]

These probiotic “superbugs” must survive the stomach and enter an ecosystem in which they are outnumbered $10,000$ to $100,000$ to one by the existing microbiome.

The viability of probiotic bacteria decreases:

(a) with time in the cooler (left)
(b) with passage through the GIT (right)

**Shelf Life of Probiotic Yogurt**

- Blue = Bifidobacteria
- Red = *S. thermophiles*
- Black = *L. delbruechii*

Viability is strain dependent

[Graph showing the viability of probiotic bacteria over time]
Probiotic Delivery has Historically been through Dairy Products

**Yogurt:** Milk fermented with *Strept thermophiles*, with or without addition of *Lactobacillus* and/or *Bifidobacteria*. Probiotics are often added after fermentation. One cup (125 ml) should contain $10^6$-$10^9$ cfu/g or ml [cfu= colony forming unit] Many different strains of *Lactobacillus* are used, but the strain is not often indicated. Probiotics add “industrialized” strains that may not have the same properties as the originals stock used in experimental studies. *Who is checking?*

**Fresh cheese:** (cottage cheese and quark) have a shorter half life than yogurt; the cfu may decline by $>2$ logs per week

**Ripened cheeses:** (Emmental, Edam, Gouda, Cheddar, aged Brick, Bier Kaese) have good shelf life. 30 g (3 slices) yields $10^6$ cfu/g

**Ice creams:** Those containing *Lactobacillus* and *Bifidobacter* and have been made since 1960. The bacteria are usually added after the product is made or it is mixed with yogurt.
Non-diary Delivery of Probiotics

Non diary delivery is often in response to lactose intolerance
  15% of Caucasians of North European origin have this problem
  60-80% of Ashkenasi Jews have this problem
  90% of Asian and Native Americans are lactose intolerant

Milk allergy affects about 3% of children

There are vegetable and fruit drinks of > 20 varieties that contain probiotic bacteria

Fermented vegetables such as sauerkraut, Kimchi and fermented pickles contain probiotics

Cereals and rice in which freeze-dried probiotic cultures are added are available

Fermented sausage (Landjaeger, Salami, Prosheuto and fermented Mettwurst)

Micro-encapsulated probiotics (alginate gels, carrageenan, gelatin, etc) can be used
Classification of Probiotic Foods

- **Probiotic foods**
  - **Dairy based**
    - Acidophilus milk, Acid-whey, Ice-cream, Lassi, Cheese, Curd, Nonfermented goat’s milk beverage, Frozen synbiotic dessert, Yoghurt, etc.
  - **Non-dairy based**
    - Fermented products
    - Unfermented products

**Cereals**
- Bread & bakery products
- Health drinks/beverages
- Puddings
- Edible film on pan bread

**Fruit**
- Juices
- Puree
- Pulp
- Beverages
- Whole fruits
- Powdered fruits

**Vegetables**
- Juices
- Puree
- Beverages

**Meat & Fish**
- Dry sausages
- Sausages

**Soy**
- Frozen desserts
- Sausages
- Soy curd
- Soy yoghurt
- Soy milk drink

Fig. 1 Classification and types of probiotic foods
Antibiotics: The 20th Century Miracle Drug

Probiotic enthusiasts should take comfort because there is a precedent for converting folklore to science

B.C. Ancients in China, Greece and Egypt successfully used mold plasters to treat wounds
A.D. ? Wounds contaminated with fungal growth in Europe and India healed faster than when uncontaminated
1874 Sir Wm Roberts noted Penicillium used for blue cheese production prevented bacterial growth
1897 Duchesne showed mice given Penicillium and Salmonella (typhus) never developed typhoid
1928 Flemming’s contaminated Petri dish culture (see below) was the key turning point for antibiotic science
1940 Penicillin could be shown to control bacterial infections in mice (Nobel Laureates Florey & Chain )
1942 First purification of penicillin from Penicillium
1943 Moldy Mary’s cantaloupe made penicillin practical; Flemming’s Penicillium could produce just 4 units/ml
    Moldy Mary’s cantaloupe at the Northern Regional Lab of the USDA produced 50,000 units /ml
1944 Authorized for use by the military
1945 Penicillin first made available to the public

“Sterile” Staph aureus culture

Staph aureus culture contaminated with bread mold Penicillium
How do eukaryotic soil fungi survive bacterial destruction since their genome changes 2-3 logs slower than bacteria? The answer is poison!

*Streptomyces sp* from soil is the source of two-thirds of all basic antibiotics.

Antibiotics have diverse chemistries and the native forms from each group have been modified by pharmaceutical chemists. Penicillin is a β-lactamase that prevents mainly Gram positive bacteria from making a cell wall.

Erythromycin is a macrolide from *Streptomyces* that blocks peptidyl transfer.

Aminoglycosides like streptomycin from *Streptomyces* inhibits protein synthesis by complexing with membrane associated ribosomes.

Tetracycline patented in 1953 and went commercial in 1978. It blocks aminoacyl-tRNA from the ribosome. Eucaryotic cells are much less susceptible than bacterial cells.
Many more Natural Antibiotics were discovered and Modified in the Lab

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Biochemistry</th>
<th>Origin</th>
<th>Activity</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>β-lactamase</td>
<td><em>Penicillium</em></td>
<td>Broad</td>
<td>Interferes with cell wall synthesis</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Aminoglycoside</td>
<td><em>Streptomyces griseus</em></td>
<td>Broad</td>
<td>Increases membrane permeability</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Quinolone</td>
<td>Synthetic</td>
<td>Broad</td>
<td>Blocks DNA synthesis</td>
</tr>
<tr>
<td>Chloramphenical</td>
<td>Amphenical</td>
<td><em>Streptomyces</em></td>
<td>Broad</td>
<td>Interferes with protein synthesis</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Macrolide</td>
<td><em>Saccharopolyspora</em></td>
<td>Limited</td>
<td>Interferes with protein synthesis</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Glycopeptide</td>
<td><em>Streptomyces</em></td>
<td>Narrow</td>
<td>Blocks peptidoglycan synthesis</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>β-lactamase</td>
<td><em>Penicillium derivative</em></td>
<td>Broad</td>
<td>Interferes with cell wall synthesis</td>
</tr>
<tr>
<td>Doxycyline</td>
<td>Macrolide</td>
<td><em>Micromonospora</em></td>
<td>Broad</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Gentimicin</td>
<td>Aminoglycoside</td>
<td>Mostly synthetic</td>
<td>Narrow</td>
<td>Alters DNA proof-reading</td>
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<tr>
<td>Tetracyline</td>
<td>Tetracycline family</td>
<td><em>Streptomyces</em></td>
<td>Broad</td>
<td>Interferes with protein synthesis</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>β-lactamase</td>
<td><em>Cephalosporium</em></td>
<td>Broad</td>
<td>Interferes with cell wall synthesis</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Polypeptide</td>
<td><em>Bacillus subtilis</em></td>
<td>Broad ?</td>
<td>Inhibits pyrophosphate recycling</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Macrolide</td>
<td>Mostly synthetic</td>
<td>Broad</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>β-lactamase</td>
<td><em>Streptomyces cattlega</em></td>
<td>Very broad</td>
<td>Inhibits cell wall synthesis</td>
</tr>
<tr>
<td>Aureomycin</td>
<td>Tetracycline family</td>
<td><em>Streptomyces</em></td>
<td>Broad</td>
<td>Inhibits protein synthesis</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>Quinolone</td>
<td>Synthetic</td>
<td>Broad</td>
<td>DNA fragmentation</td>
</tr>
<tr>
<td>Amphicillin</td>
<td>β-lactamase</td>
<td>modified penicillin</td>
<td>Broad</td>
<td>Interferes with cell wall synthesis</td>
</tr>
</tbody>
</table>

Nearly all antibiotics are broad spectrum since there is little incentives to produce bacteria-specific forms

Tetracyclines and fluoroquinolines are banned from use as AGP in most countries. [See Session 4; Lecture 2]
The Impact of Antibiotics

600 AD  *Yersinia pestis* (Bubonic plague) killed > 100 million or twice as many as Hitler
1400 AD  *Yersinia* killed one-fourth of the population of Europe and > 10 million in India
1865  More than half of the 700 thousand soldiers who died in the civil war died from bacterial infection
1918  In WW I “More soldiers died from typhus (*Salmonella*) and diarrhea (*E. coli*) than from bullets”
       [The actual number is 4 million versus 2 million just among the allies]
1943  War Production Board allows mass production of penicillin
1945  Data from WW II (*after penicillin*) is difficult to determine since most of the 60-80 million non-holocaust victims were civilians. In the US military it was 8%.
1956  Of the 1.2 million who died in Korea, < 2% was due to bacterial infection

Pest saule

Scupture in Dachau

Bergen-Belzen

“Antibiotics caused the militaries to loose half of their arsenal” ; a fact not lost on the Japanese, Russians or Americans!!
No Free Lunch: “The Micobial Empire Strikes Back”

The “Antibiotic Winter?”

Newton: “For every action there is an equal an opposite reaction”.

Tom Frieden (CDC): “Each year two million Americans get infection from antibiotic-resistant bacteria and 23,000 die” (25,000 in the UK)

Inf. Dis. Soc.: “The antibiotic pipeline does not match bacteria’s ability to develop resistance. It is estimated that 10 million will die per year in 2050 from these ‘superbugs’”

### Historical Evidence of Bacterial Resistance

<table>
<thead>
<tr>
<th>Year</th>
<th>Antibiotic</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1942 (Rammelkamp et al)</td>
<td>Penicillin</td>
<td>Staph. aureus</td>
</tr>
<tr>
<td>1963 (Jerons et al)</td>
<td>Methicillin</td>
<td>Staph. aureus (MRSA)</td>
</tr>
<tr>
<td>1967 (Hansmn et al)</td>
<td>Penicillin</td>
<td>Strept. pneumoniae</td>
</tr>
<tr>
<td>1988 (Uttley et al)</td>
<td>Vancomycin</td>
<td>Enterobacteria (VRE)</td>
</tr>
<tr>
<td>1997 (?)</td>
<td>Vancomycin</td>
<td>Enterococcus (turkey farmers)</td>
</tr>
<tr>
<td>1999 (Smith)</td>
<td>Vancomycin</td>
<td>Staph aureus</td>
</tr>
<tr>
<td>2001 (Many investigators)</td>
<td>Quinolines</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td></td>
<td>Cephalosporin</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td></td>
<td>Tetracyline</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td>2013 CDC Report</td>
<td>(five or more)</td>
<td>Enterobacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridium difficile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neiserria gonorrheae</td>
</tr>
</tbody>
</table>
Is Antibiotic Resistance caused by Over-prescription?

One-third to one-half of all outpatients subscriptions are unnecessary
Greatest misuse is in pediatrics in the treatment of the common cold [a rhinovirus]
The pattern of seasonal prescription rates reflect this misuse; a 59% increase during winter months

Antibiotics are not mutagens but they are selection agents
Antibiotic resistance was known in the 1930s before antibiotics were used in medicine
Bacterial genes mutate at a rate of $10^{-9}$ per generation and this does not change in the presence of antibiotics
Fungi and other bacteria have been making antibiotics before vertebrates appeared on earth.

80% of all antibiotics sold are for use as antibiotic growth promoters (AGP) in animal production (Session 4; Lecture 2)
The voice of medical science is often drowned out by the agricultural lobby
Are Antibiotics Responsible for Creating 20th Century Diseases?

IBD & Relative Prescription Frequency

Red = Relative Prescription Frequency
Blue = IBD Incidence

Incidence of Inhaled Allergy, Food Allergy and Rate of Antibiotic Prescriptions

Blue = Inhaled Allergies
Purple = Food Allergy
Red = Antibiotic Prescriptions

Colon Cancer versus Antibiotic Prescriptions Rate

Blue = Colon cancer
Red = Relative Prescription Levels

Incidence of Asthma, Autism and Relative Prescription Frequency

Red = Relative Prescription Levels
Green = Asthma
Blue = Autism
The Increase in 20\textsuperscript{th} Century Disease is Geographically Non-random

Crohn’s Disease Example

Data for other forms of IBD show a similar pattern.
Geographical Variations and 20\textsuperscript{th} Century Diseases

**IBD and Antibiotics**

Sweden has among the highest levels of IBD but with one of the lowest usage rates of antibiotics
Greece, Belgium and Italy have only modest levels of IBD but among the highest usage of antibiotics
[28 DDD versus < 10 DDD for Sweden, Germany and Netherlands]
South Africa is the only African country with IBD and with a history of modern medicine and antibiotic use
IBD is almost absent in South America were antibiotic use is very low

**Geographical Variation in the “20\textsuperscript{th} Century Diseases”**

Asthma is highest in developed countries and lowest in Asia, Eastern Europe and Africa

Asthma is higher in Mexicans born in America than in non-US born Mexicans living in America

Allergy is highest in developed countries and lowest in countries that rely on traditional agriculture

Autism 36% higher in the US than in the UK

Obesity doubled in three decades in the USA and especially in low income/low economic groups

Obesity is ~ 30% higher in the US than in Canada
What else has Changed since the Introduction of Antibiotics?

Disappearance of Polio, Diphtheria and Measles

Seven-fold increase in emerging viral pandemics

The Obesity Epidemic

Incidence of asthma in children

Acid reflux Disease

McDonalds’, Burger King & Pizzerias

Jet Airplanes

Use of Cannabis

The “Pill”

Migration from farm to urban environment and decrease in family size especially in Europe and near Asia
What is the Role of the Microbiome in 20th Century Diseases?

Do increases in diseases parallel alterations of the microbiome?

Does the disease cause the alteration of the microbiome?

Does the change in the microbiome cause the disease?

Does the inappropriate use of antibiotics facilitate increase in disease?

Suggested read: *Missing microbes* by Martin Blaser, MD, New York University [Picador]