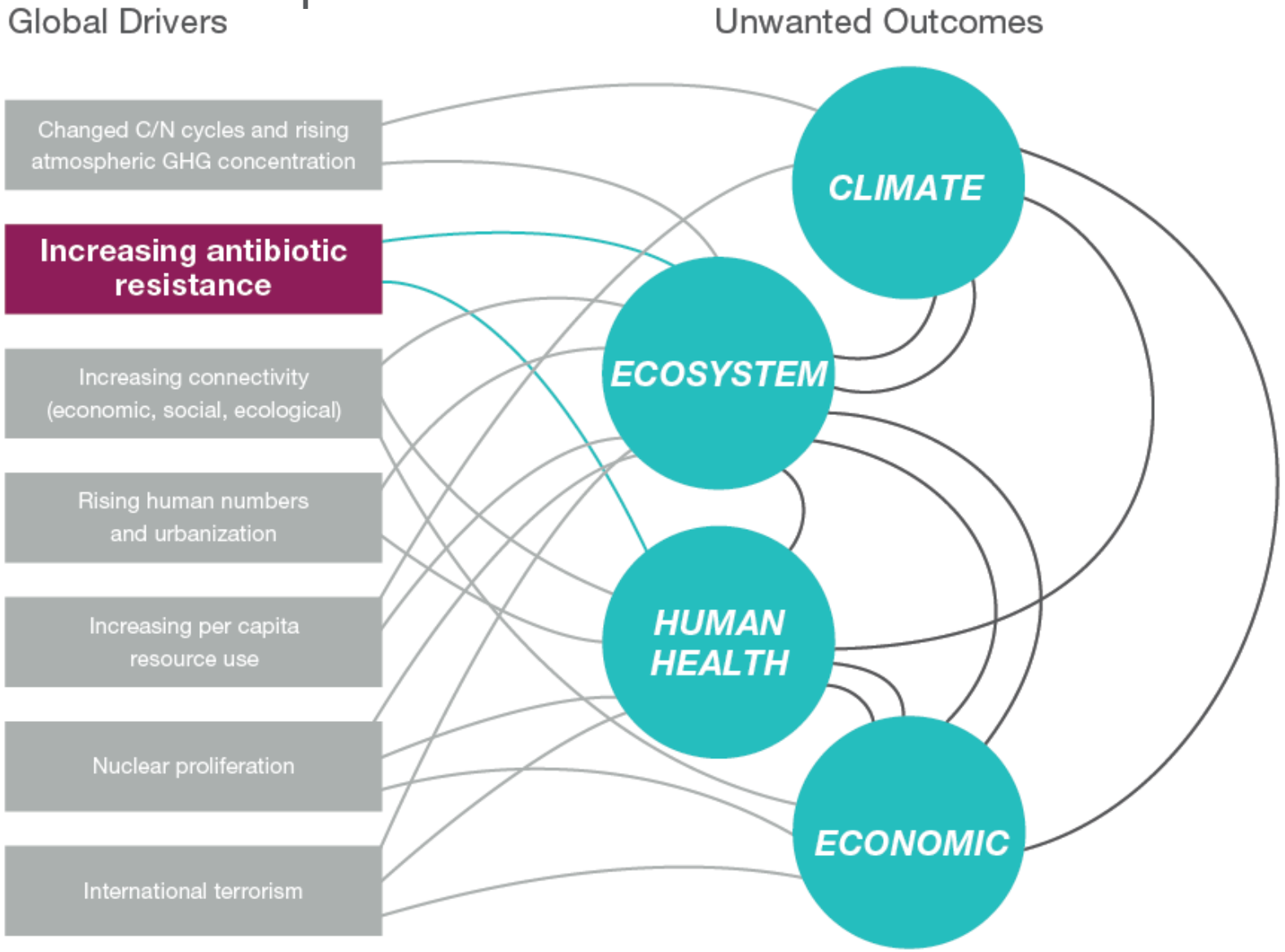
Don’t Take that Antibiotic. . . You May Get Fat?

The Science of Healthy Microbiome

Norman Moore, PhD

What do you think are the top 7 threats to the human race?

One of the top 7 issues that threatens the human race



Source adapted from: Science, Vol 325, September 2009
Available at: http://www.sciencemag.org/content/325/5948.cover-expansion

TEST
TARGET
TREAT™

Antimicrobial Resistance

Inpatient Settings

One in every three patients will receive two or more antibiotics in the course of their hospital stay

Of the patients receiving antibiotics, three out of every four will receive unnecessary or redundant therapy, resulting in excessive use of antibiotics

CDC – Get Smart Campaign

Outpatient Settings

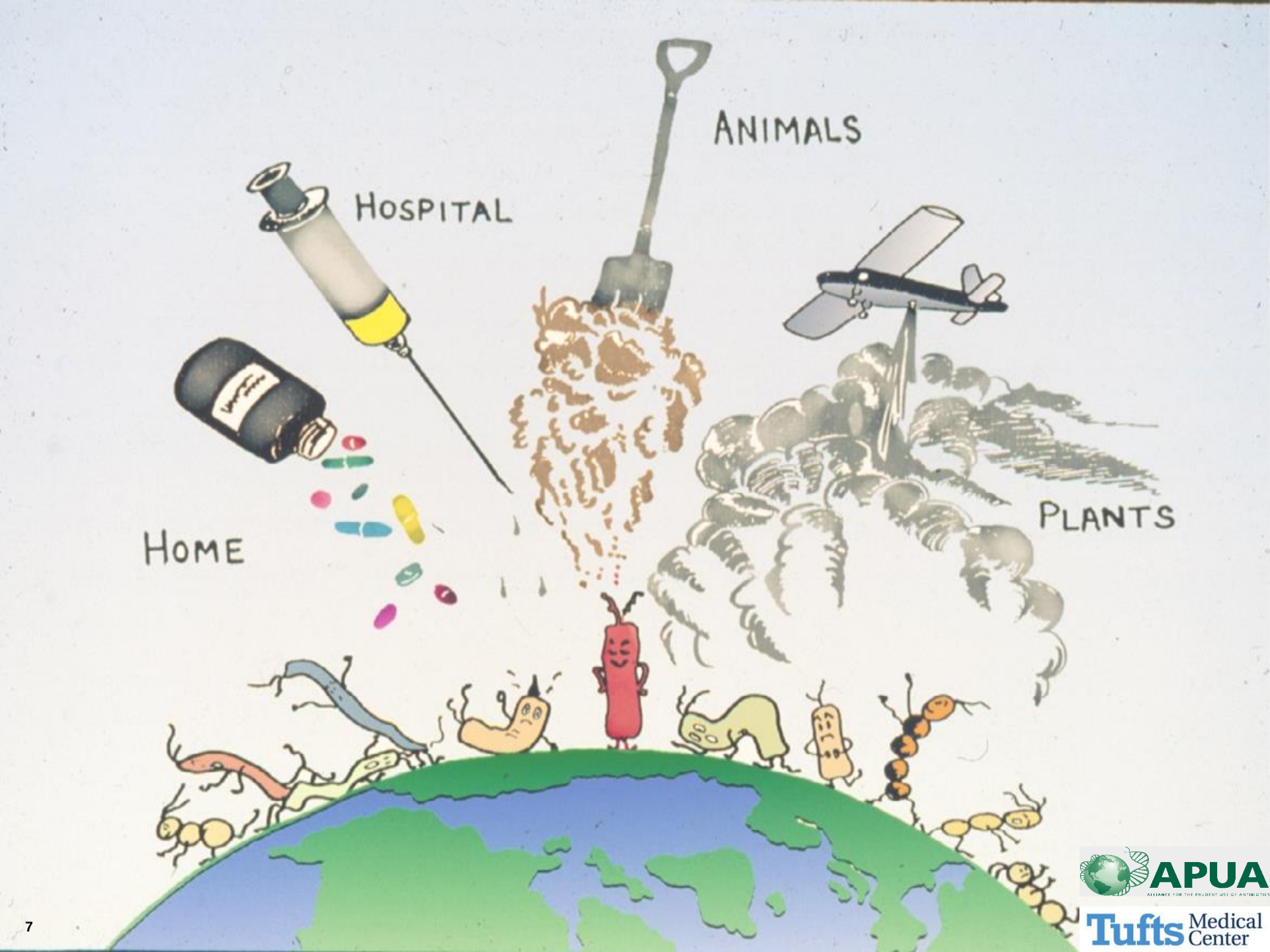
Each year, tens of millions of antibiotics are prescribed unnecessarily for upper viral respiratory infections

Antibiotic use in primary care is associated with antibiotic resistance at the individual patient level

The presence of antibiotic-resistant bacteria is greatest during the month following a patient's antibiotics use and may persist for up to 1 year

CDC – Get Smart Campaign

Test Target Treat model



APUA
ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS

Tufts Medical Center

What percent of antibiotics made in this country goes into animal feed?

What percent of antibiotics made in this country goes into animal feed?

80%

Why Use Antibiotics With Animals?

Therapeutic

- If they have a disease

Prophylactic

- Before an expected exposure or immediately after an exposure, but prior to illness

Non therapeutic

- No issue of disease prevention

Non Therapeutic Use

Promotion of weight growth

- Can be given in sub-therapeutic doses in food and water

Livestock can become resistant

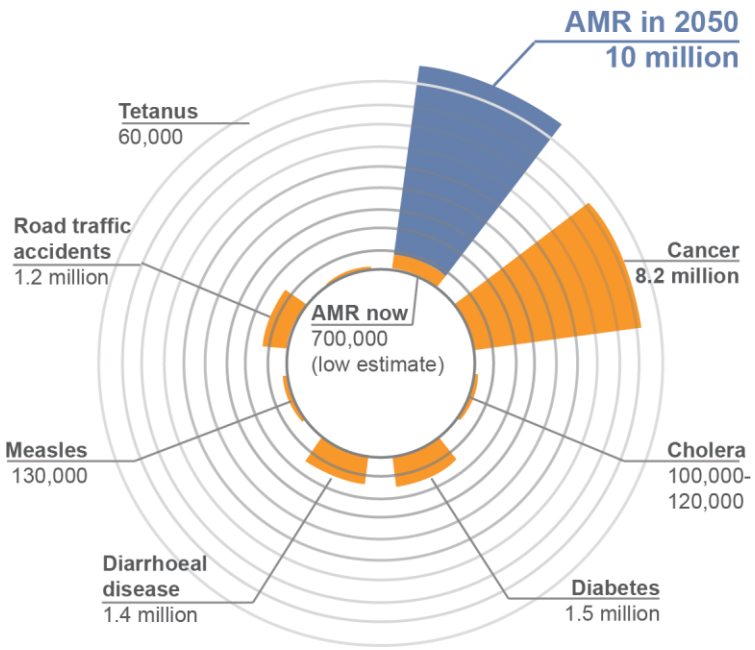
- Through feces, spread to other animals and water supply

Antibiotics found in meat

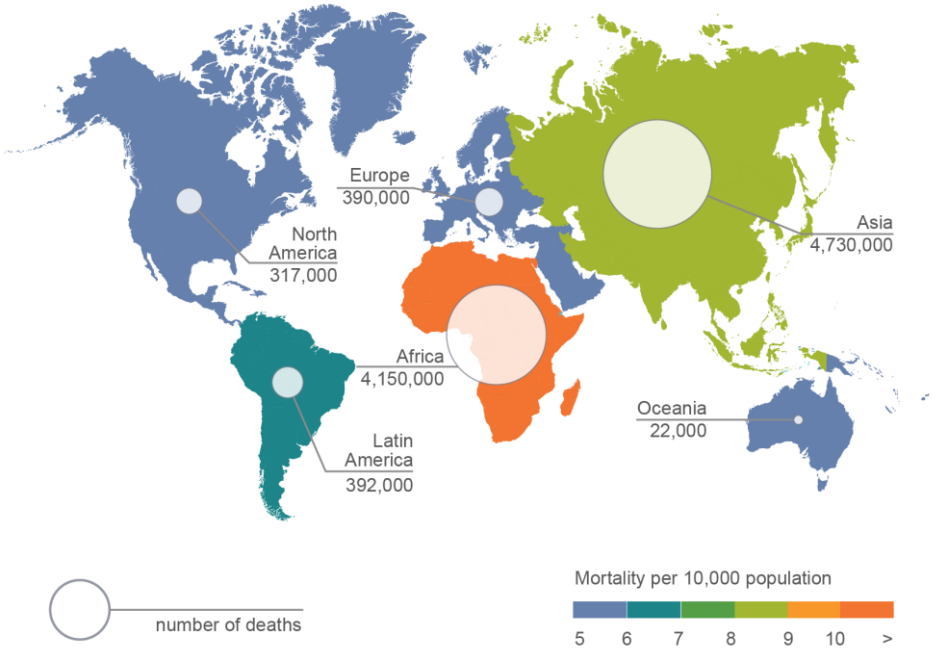
- Penicillin
- Tetracycline
- Sulfanamides
- Neomycin
- Gentamycin
- Streptomycin

AMR: If We Don’t Take Action Now

Deaths attributable to AMR every year
compared to other major causes of death



Deaths attributable to AMR every year
by 2050



Study on CAP Patients and Therapy

Retrospective study on 175 CAP patients in New York

- Exclusion criteria
  - Hospitalization ≥ 2 days within 90 days
  - Residence in nursing home
  - Prior isolation of MDR organism

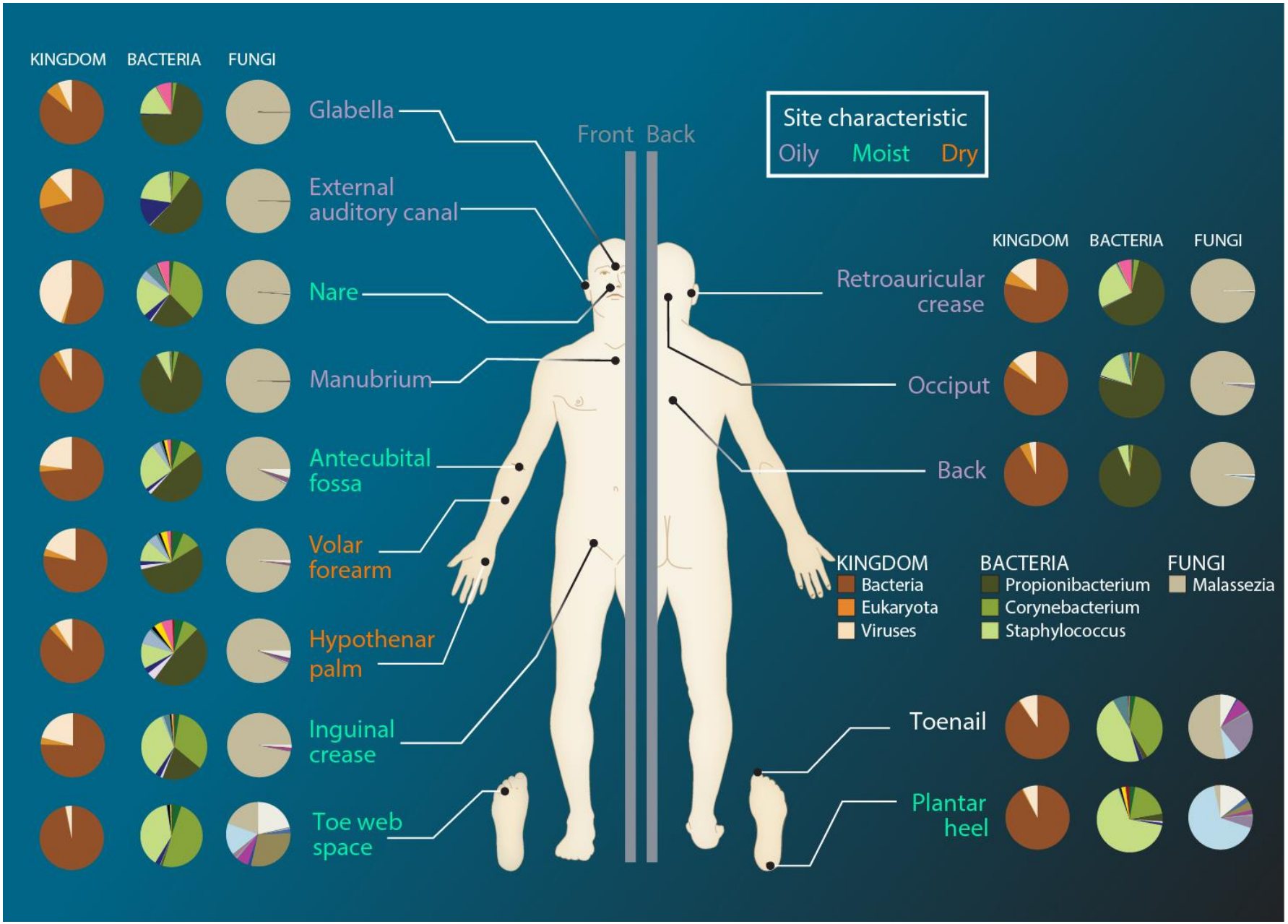
Rate of multidrug resistant organism detected within 90 days

- 15% patients on fluoroquinolone
- 4% of patients on cephalosporin/macrolide

What is a Microbiome?

Basic Definitions

Microbe	• Microorganisms such as bacteria, fungi, viruses
Microbiome	• The community of microbes
Biofilm	• Community of microbes on a surface



Prebiotics & Probiotics

Both can change gut flora

Prebiotics

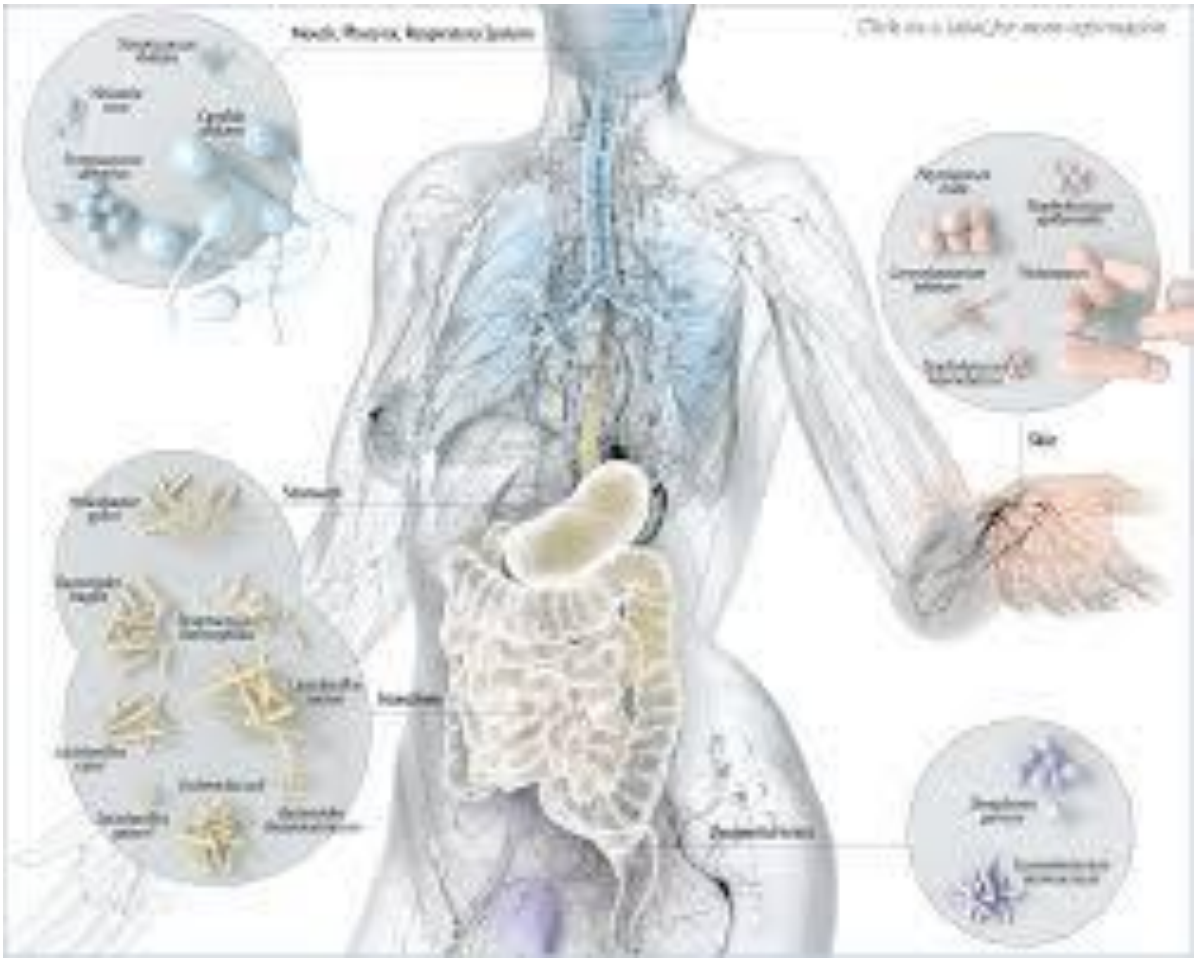
- Food ingredient that can't be digested by a person, but can by microorganisms
- Shown to increase the genus
- Prebiotics have been shown to increase the genus *Akkermansia* which is thought to help maintain body weight
  - Publicized that there is a human trial giving obese people more *Akkermansia*
- Possible improvements to glucose tolerance, lower blood triglycerides, and body fat in rat studies

Probiotics

- Bacterial cultures themselves
- Can be limited in scope
- Not well regulated
- Giving healthy people vs. sick?

We are Outnumbered!

10 trillion	• The number of human cells in a body
100 trillion	• The number of microorganisms in a body
20,000	• Estimate of human genes
9 million	• Estimate of bacterial genes



Gut Microbiome aka “The Second Brain”

Over 1,000 species in gut

10^{12} microbes per gram luminal content (1,000,000,000,000)

60% of fecal weight

How Do You Develop A Microbiome?

In the womb, a baby is sterile

- Initial exposer is through the mother's bacteria
  - Vaginal, fecal, and skin
- Breast feeding
  - Establish the appropriate gut colonization
  - Can be disrupted with bottle feeding and/or antibiotic administration

Gut Microbiome Interactions

Educating the immune system

- Segmented filamentous bacteria (SFB) essential in development of T_h17 cells
- *Bacteroides fragilis* converts pro-inflammatory $CD4^+$ T cells into Tregs

Regulation of the immune system

Microbe-microbe interaction

- Produce microcins, bacteriocins, and colicins to stop invading bacteria and not injure host
- Cooperate on food
- Horizontal gene transfer

Christina Ohland and Christian Jobin. Microbial activities and intestinal homeostasis: a delicate balance between health and disease. Cell Mol Gastroenterol Hepatol. 2015: 1: 28-40.

Microbiome benefits

Help digest food

Make vitamins – B2, B12, K

Barrier from pathogens

- *C. difficile* in gut

Attacking pathogens

- *E. coli* can produce bacteriocines

Modulate innate and adaptive immunity

Gut Diversity with Antibiotics

Question

- How much can a single dose of antibiotics affect the gut microbiome

Experiment

- Antibiotic-naïve children in Niger
- Half got a single dose of oral azithromycin
- Half got a placebo

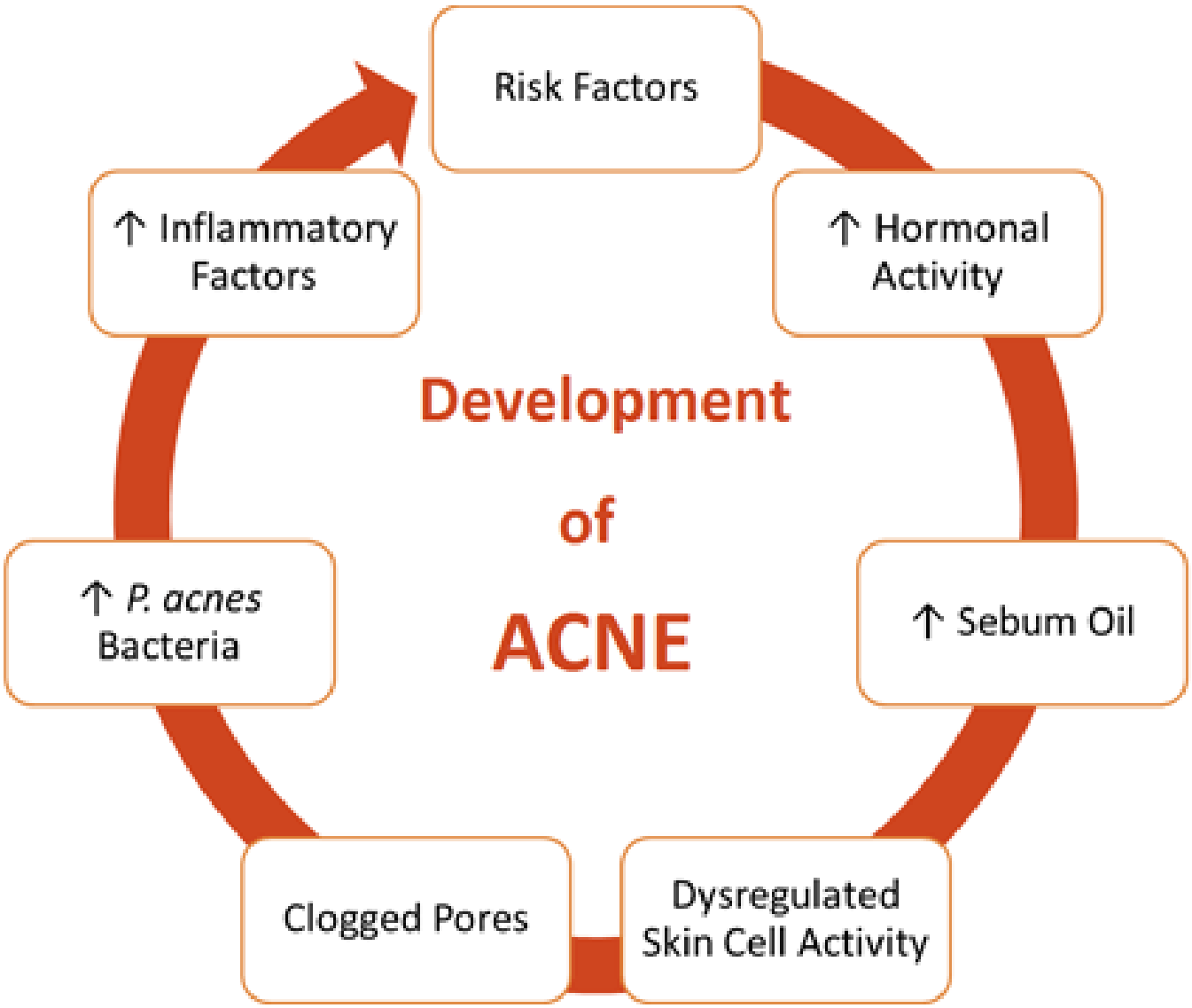
Results

- At 5 days, azithromycin-treated group had significantly less gut diversity

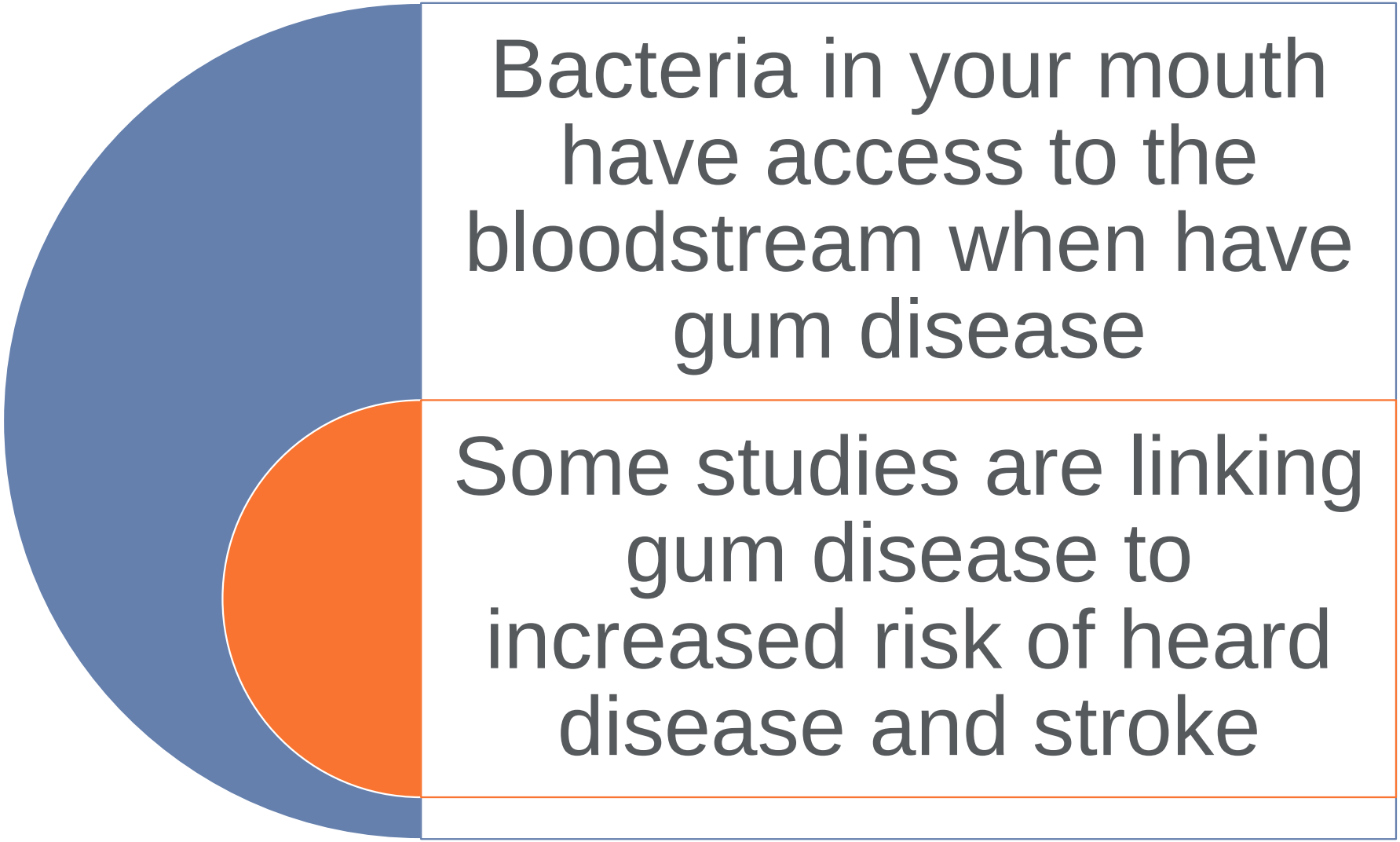
Doan, Thuy, AM Arzika, KJ Ray, SY Cotter, J Kim, R Maliki, L Zhong, Z Zhou, T Porceo, B Vanderschelden, J Keenan, and TM Lietman. Gut microbial diversity in antibiotic-naïve children after systemic antibiotic exposure: a randomized controlled trial. CID. 2017: 1147-1153

Diseases

Acne Cycle


Risk Factors
↑ Hormonal Activity
↑ Sebum Oil
Dysregulated Skin Cell Activity
Clogged Pores
↑ P. acnes Bacteria
↑ Inflammatory Factors
Development of ACNE

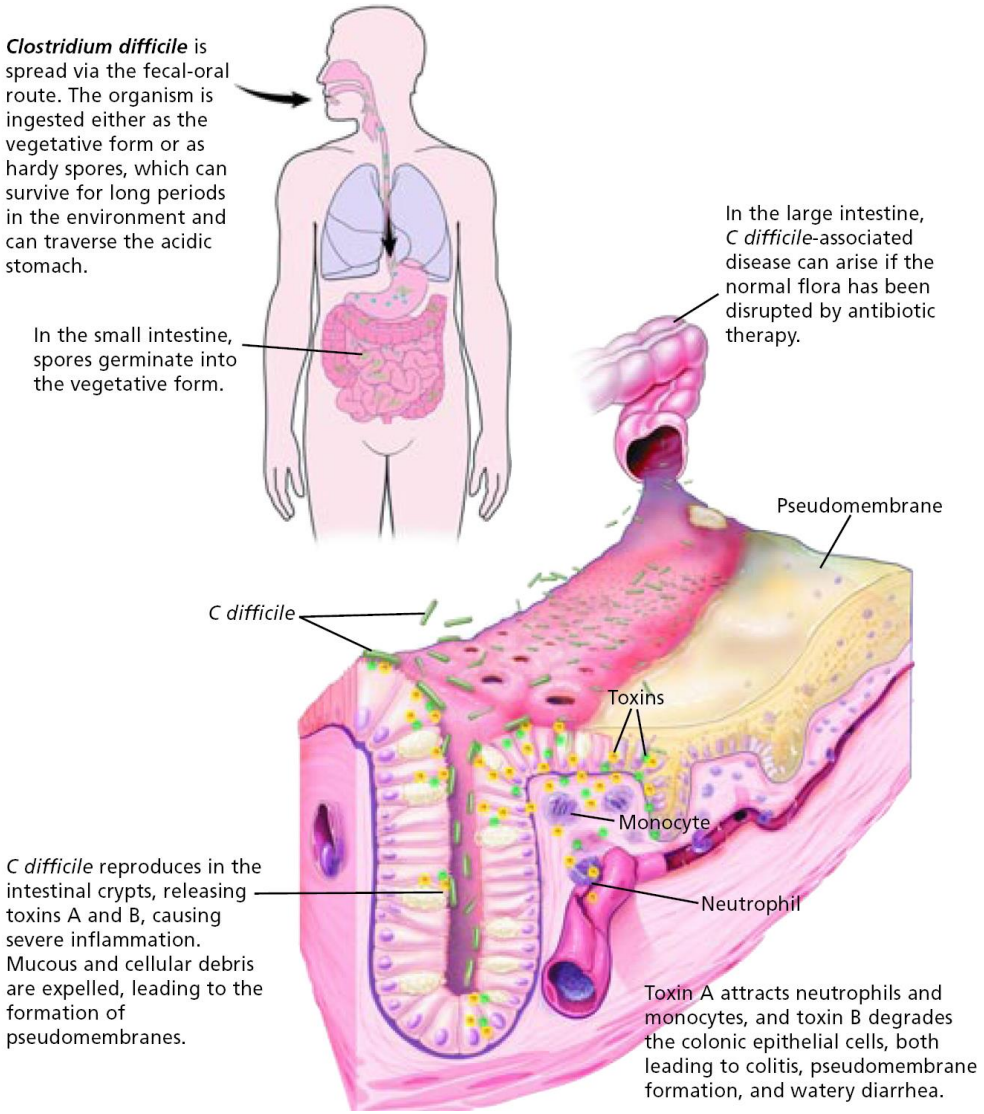
Mouth Flora and Other Diseases



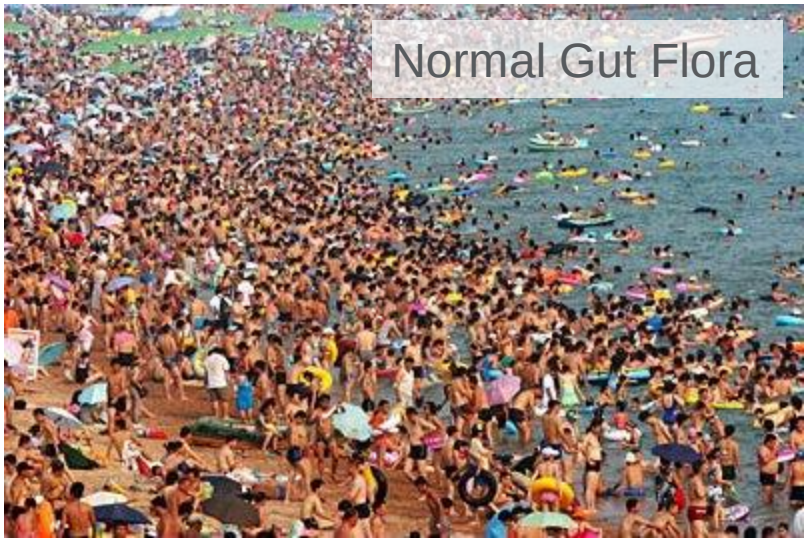
Clostridium difficile - Background

- Gram positive spore former – the most common cause of healthcare-associated diarrhea
- Spread by health care workers - spores difficult to eradicate
- Causes 25% of antibiotic associated diarrhea and 90-99% of pseudomembranous colitis
- Disease is caused by the toxins the organism produces

Pathogenesis of CDAD



Antibiotic-Associated Diarrhea: Life's a Beach with *C. difficile*



Community-Associated

Recent studies suggest 20-45% of CDI cases are CA-CDI

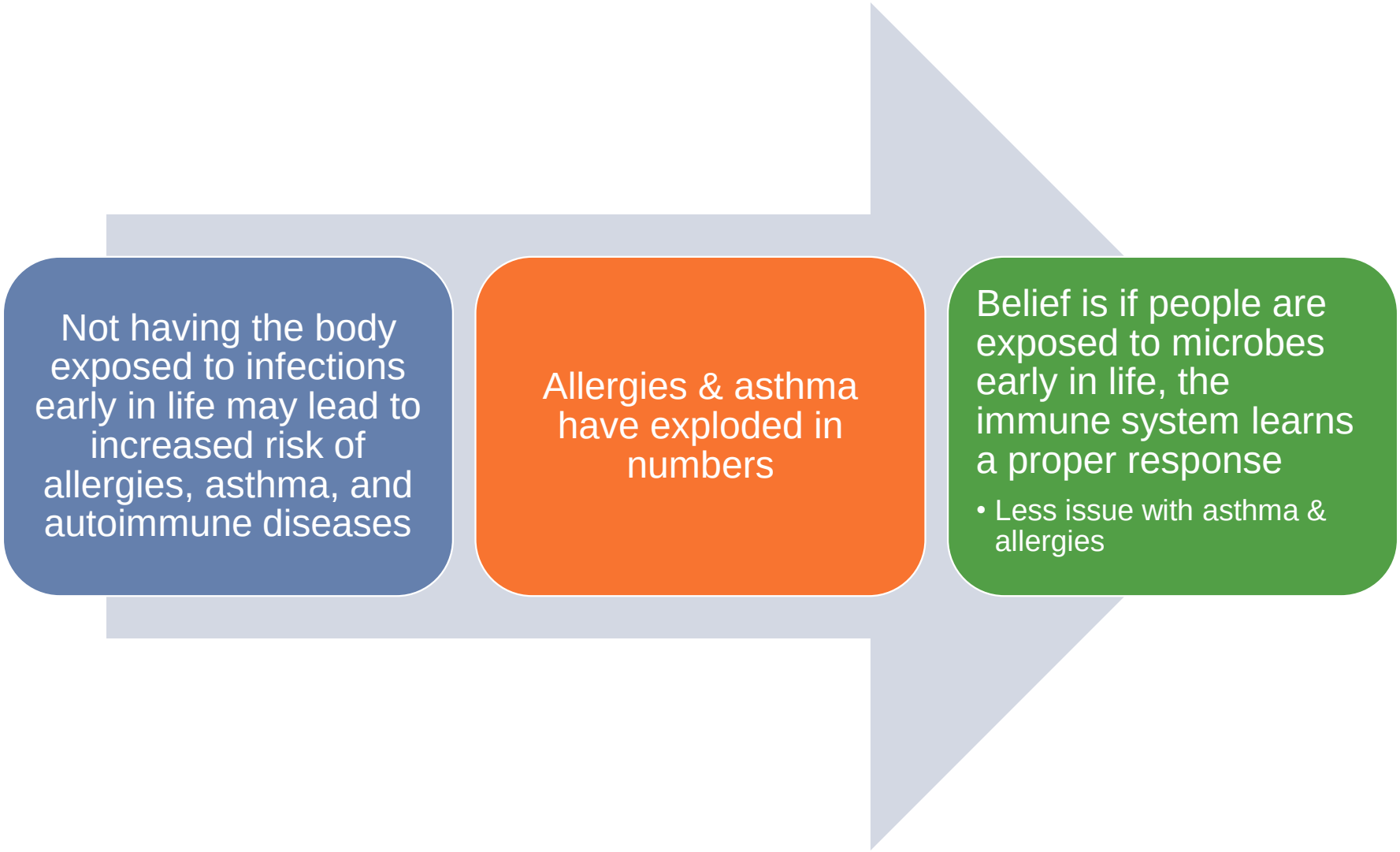
- Approximately 22% not exposed to antimicrobial agents in 90 days prior
- Usually present with less severe symptoms
- Tend to be younger and female compared to healthcare-associated
  - One study had age 50 vs 72 years and 76% female vs. 60%

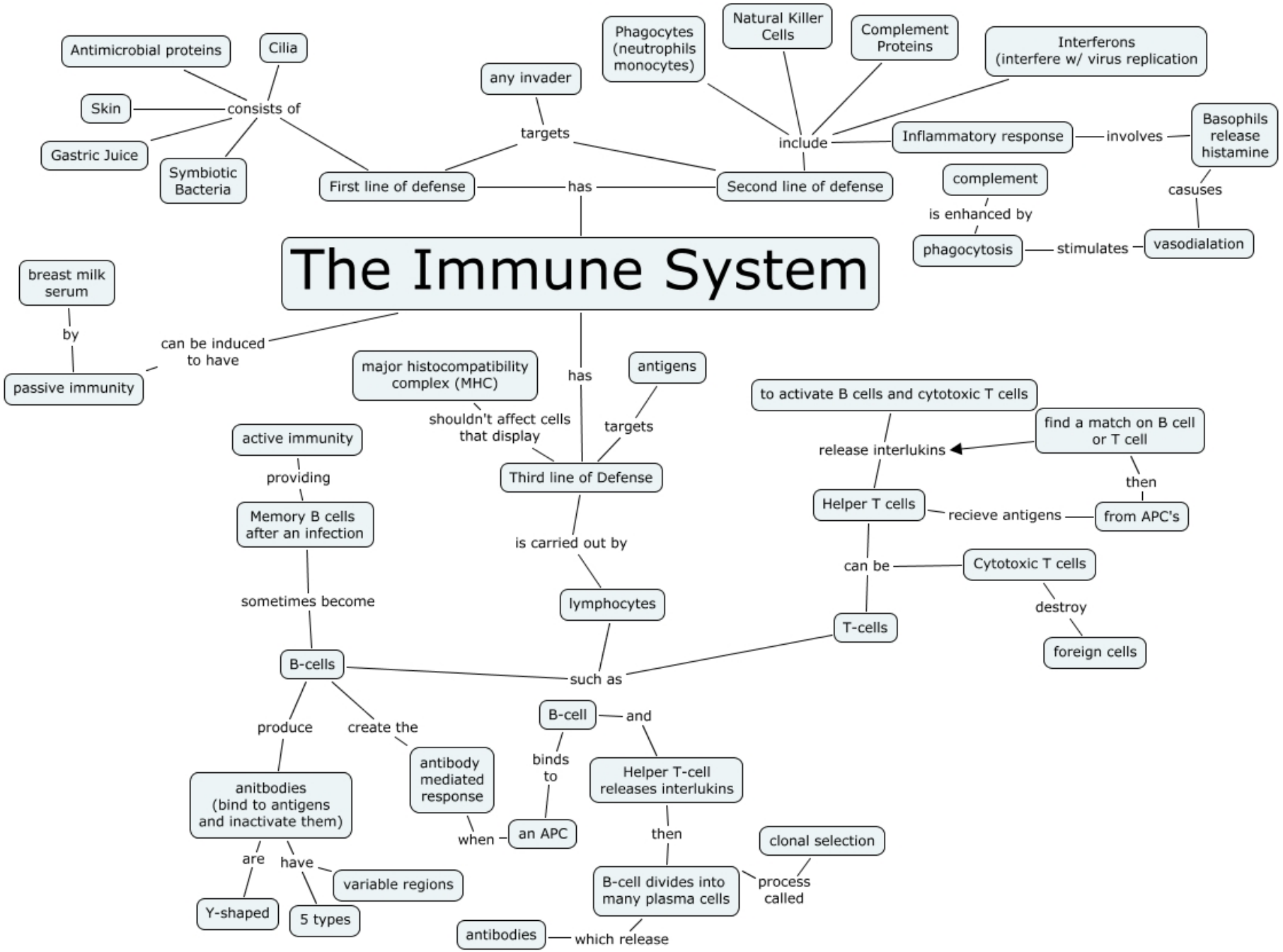
Risk factors

- Antibiotic exposure – highest first 30 days, but higher risk continues to 60 days & not return to baseline until 150 days
- Outpatient visits
- Contact with *C. difficile* patient
- Proton pump inhibitors?
- Animals? *C. diff* can also colonize calves & pigs and dogs & cats?

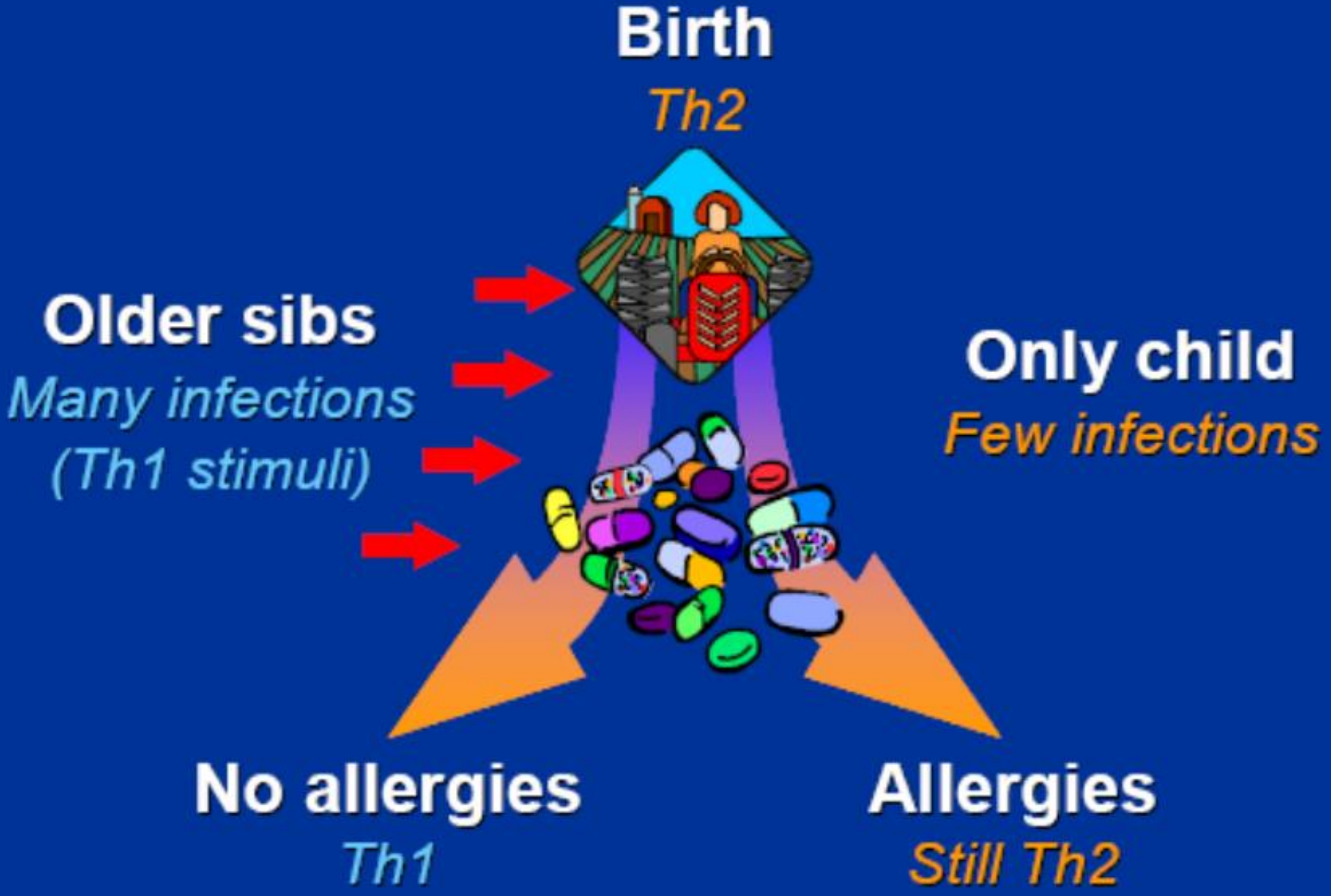
Hygiene Hypothesis & Allergies and Autoimmune Disease

The Hygiene Hypothesis



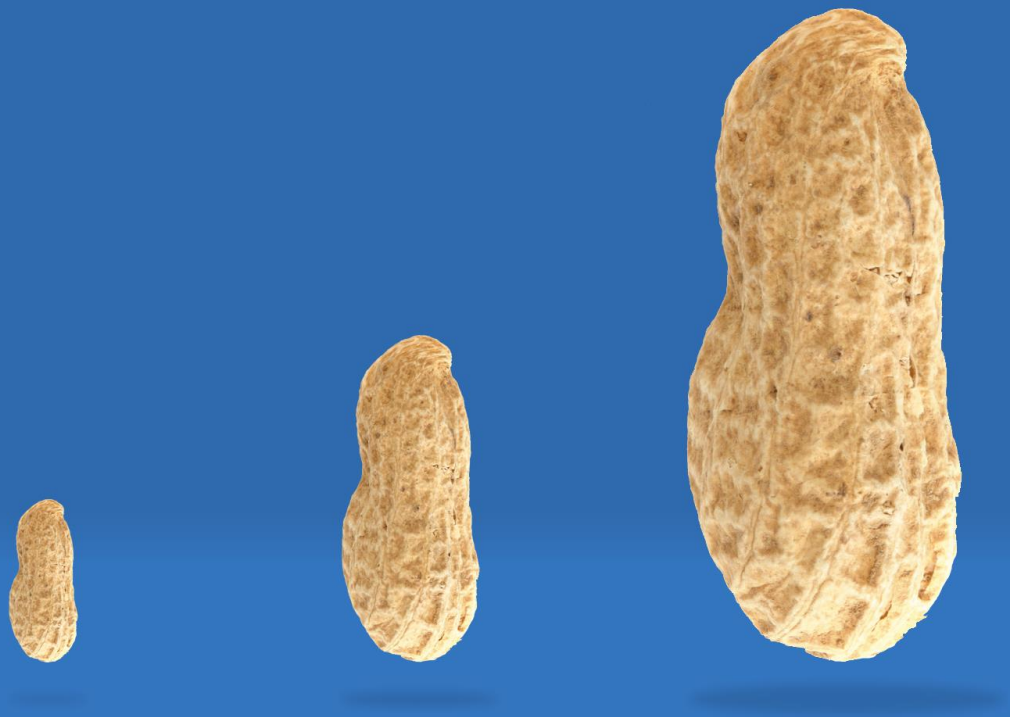

The Immune System
Antimicrobial proteins
Cilia
Skin
consists of
Gastric Juice
Symbiotic Bacteria
First line of defense
any invader
targets
Phagocytes (neutrophils monocytes)
Natural Killer Cells
Complement Proteins
Interferons (interfere w/ virus replication
include
Second line of defense
has
Inflammatory response
involves
Basophils release histamine
casuses
vasodialation
complement
is enhanced by
phagocytosis
stimulates
breast milk serum
by
passive immunity
can be induced to have
major histocompatibility complex (MHC)
shouldn't affect cells that display
has
antigens
targets
Third line of Defense
is carried out by
lymphocytes
active immunity
providing
Memory B cells after an infection
sometimes become
B-cells
such as
to activate B cells and cytotoxic T cells
release interlukins
find a match on B cell or T cell
then
Helper T cells
recieve antigens
from APC's
can be
Cytotoxic T cells
destroy
foreign cells
T-cells
produce
create the
anitbodies (bind to antigens and inactivate them)
are
have
Y-shaped
5 types
variable regions
antibody mediated response
when
an APC
B-cell
and
binds to
Helper T-cell releases interlukins
then
B-cell divides into many plasma cells
clonal selection
process called
antibodies
which release

The Hygiene Hypothesis



According to the American Academy of Allergy, Asthma and Immunology:

Peanut allergies alone have tripled from 1997-2008

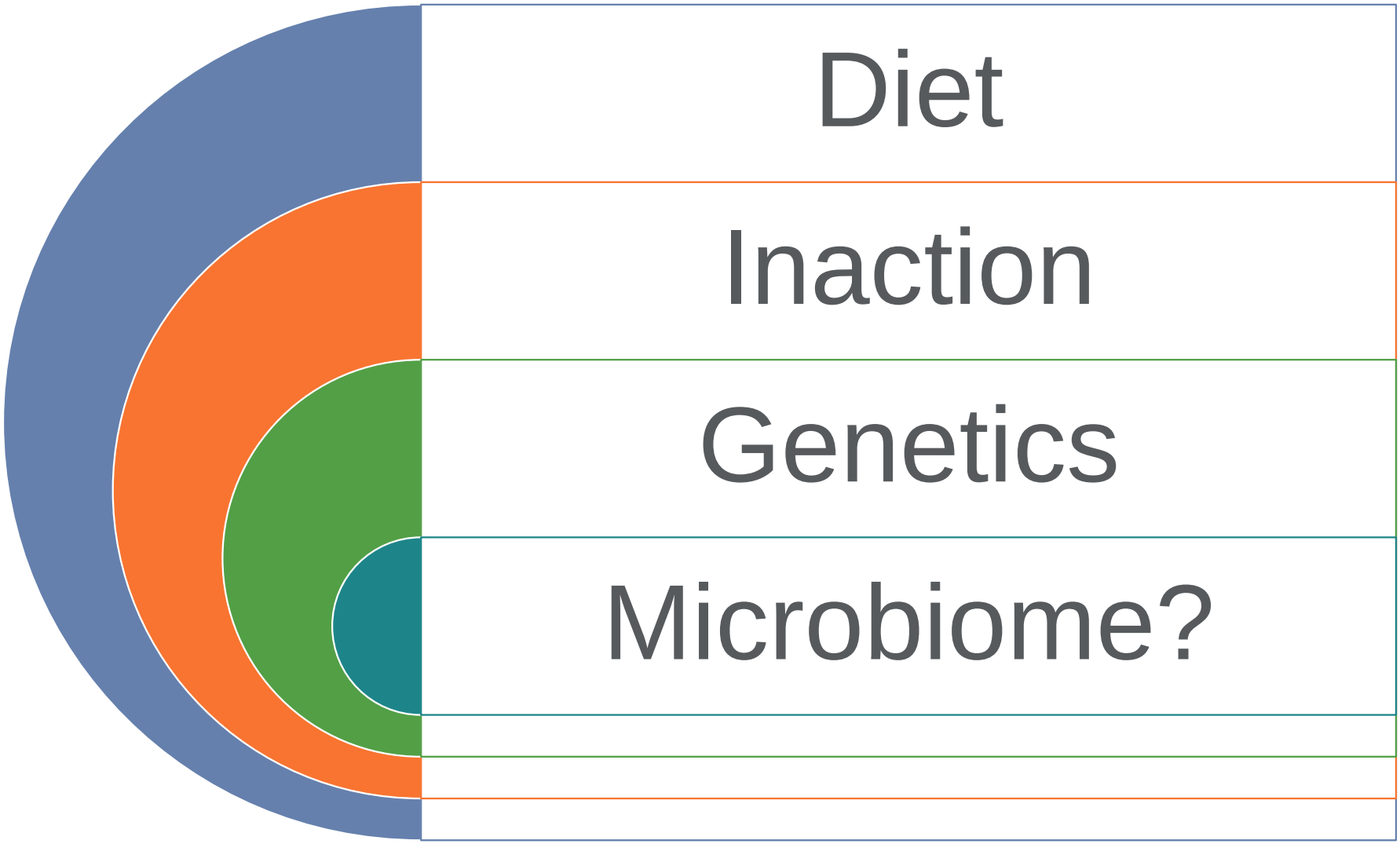


MyKidsFoodAllergies.com

http://www.mykidsfoodallergies.com/hygiene-hypothesis-clean-causing-food-allergies/

Obesity

Things That Lead to Obesity



Mouse Experiments

In 2005, shown that obese mice and lean mice had differing microflora in their gut

Gut implantation

- If take gut flora from an obese mouse to a germ-free mouse, can make the germ-free mouse obese, depending on the diet
- The same thing happens when taking gut flora from an obese person
- Control is non-obese mouse

Additional research has shown that a high fat diet can change bacterial diversity in the gut.

Rood, Jenny. The Scientist. November 1, 2015.

Mouse/Human Experiments 1

Twins

- Comparison of both thin or both obese
- Thin twins have more diverse microflora

Raise genetically identical “humanized” mice in germ-free environment

- Carry functioning human genes/tissues
- Give one mouse intestinal flora from obese twin and another from thin
- Given same diet
- Mouse with diet from obese person gained more fat

Same experiment – move mice to shared cage

- Both remained lean
- Fecal/oral transmission

Mouse/Human Experiments 2

Transferring bacteria

- Moved 54 strains from lean to obese community gave shift to lean
- Moved 39 strains and wasn't effective

Transfer bacteria & then give "Western" diet

- High fat, low in fruits, vegetables, and fiber
- Result is obese mice stayed obese even when living with thin mice
- Diet didn't allow change in gut flora

Antibiotics in low doses

- Mice had 15% more body fat than controls with less microbial diversity

Findings

Microbes that are best at getting nutritional value from high fat foods and then stimulating the storage of that food as fat are selected for.

High diversity gut flora is linked to better health

Establishing a Gut Microbiome in Babies

Formula-fed & C-section babies have higher risk of obesity and diabetes than breastfed and vaginal

- Newborns swallow bacteria as they transverse the birth canal
- Breast milk may have substances that better nurture beneficial bacteria & potentially limit colonization of harmful bacteria

One thought – Add the bacteria they may be missing

- Clinical trial in Puerto Rico on C-section babies
- Swabs babies with gauze from vaginal fluid of babies
- Track health/weight compared to other C-section

Cancer

Microbes Specifically Associated with Cancer

Human Papilloma Virus (HPV)	• Cervical cancer
H. pylori	• Ulcers • Stomach & esophageal cancer
Fusobacterium	• Colon cancer
Hepatitis B & C	• Liver cancer
Epstein-Barr Virus (EBV)	• Lymphomas

Does Breast Tissue Have a Microbiome

Study done on 18 to 90 year old women

- Some have lactated and others haven't
- Different areas of the world
- If sample submitted for cancer, sample taken 5 cm away from tumor

Canadian samples

- Bacillus (11.4%)
- *Acinetobacter* (10%)
- *Enterobacteriaceae* (8.3%)
- *Pseudomonas* (6.5%)
- *Staphylococcus* (6.5%)
- *Propionibacterium* (5.8%)
- *Comamonadaceae* (5.7%)
- *Gammaproteobacteria* (5%)
- *Prevotella* (5%)

Irish

- *Enterobacteriaeceae* (30.8%)
- *Staphylococcus* (12.7%)
- *Listeria welshimeri* (12.1%)
- *Propionibacterium* (10.1%)
- *Pseudomonas* (5.3%)

Urbaniak, et al. Microbiota of human breast tissue. Applied and Environmental Microbiology. 2014. 80: 3007-3014.

How Is the Breast Microbiota Established?

How do bacteria get there?

- From skin through nipple-areolar orifices
- Hands
- Translocation from gut
- Oral – breast feeding or sexual

Breast tissue has distinct environment

- pH
- Oxygen levels
- Diet?

Breast Cancer

Statistics

- Leading cause of cancer death among women
- 70% of breast cancers are from women of average risk
- About 292,130 women and 2,350 men diagnosed with breast cancer each year
- Every 2 minutes, a new case is diagnosed and 13 minutes, a woman dies from breast cancer

The Microbiome of Breast Cancer

Breast tissue obtained from sterile surgery has its own unique microbiome

- Not the same as from overlying breast skin

Breast microbiome different between benign and malignant disease

- Malignant disease had enrichment of *Fusobacterium*, *Atopobium*, *Gluconacterobacter*, *Hydrogenophaga*, and *Lactobacillus*
- Hypothesized *Fusobacterium* secretes virulence factors and leads to a pro-inflammatory environment that can potentially promote carcinogenesis

Hieken et al. The Microbiome of aseptically collected human breast tissue in benign and malignant disease. Scientific Reports. DOI: 10.1038/srep30751.

The Big Question

Can a change in the microbiome reduce the risk of cancer?

- Restore the “appropriate” microbiome and eradicate causative organism?
- Looking at *Fusobacterium* and colorectal carcinoma

Rheumatoid Arthritis

General Information

Autoimmune & inflammatory disease

- Usually joints, such as hands, wrists, and feet
- Can affect organs such as lungs, heart, and eyes

Usually associated with

- Age
- Sex
- Genetics
- Obesity
- Smoking

Rheumatoid Arthritis and Microbiome

Microbiome Change

- *Prevotella copri* highly associated with new-onset rheumatoid arthritis
  - Identified specific genes correlated with disease
- Reduction in *Bacteroides*

Other evidence

- Mice with mutations that increase risk of arthritis don't get it if kept in sterile conditions
- Exposed to certain species normally found in gut, then develop joint pain

Scher et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthtritis.

eLife 2013;2 e01202. DOI: 10.7554/eLife01202

Autism

Autism Basics

Been described as a medical condition since 1943

Early rates were roughly 1 in 5000 and now are 1 in 68.2

Boys are four to five times greater chance to have it

Potential causes

- Genetic factors?
- Maternal immune factors?
- Prenatal or environmental toxicity?
- Metabolic derangement?
- Gastrointestinal and dietary factors?

Buie, Timothy. Potential etiologic factors of microbiome disruption in autism. Clinical Therapeutics. 2015. 37: 976-983.

Autistic Children and Gastrointestinal Issues

Data suggests autistic children have same GI issues as usual pediatric community, but at a larger frequency

- Diarrhea
- Constipation
- Acid reflux

Is this due to altered eating patterns?

Is There Evidence Supporting the Connection Between Autism and an Altered Microbiome?

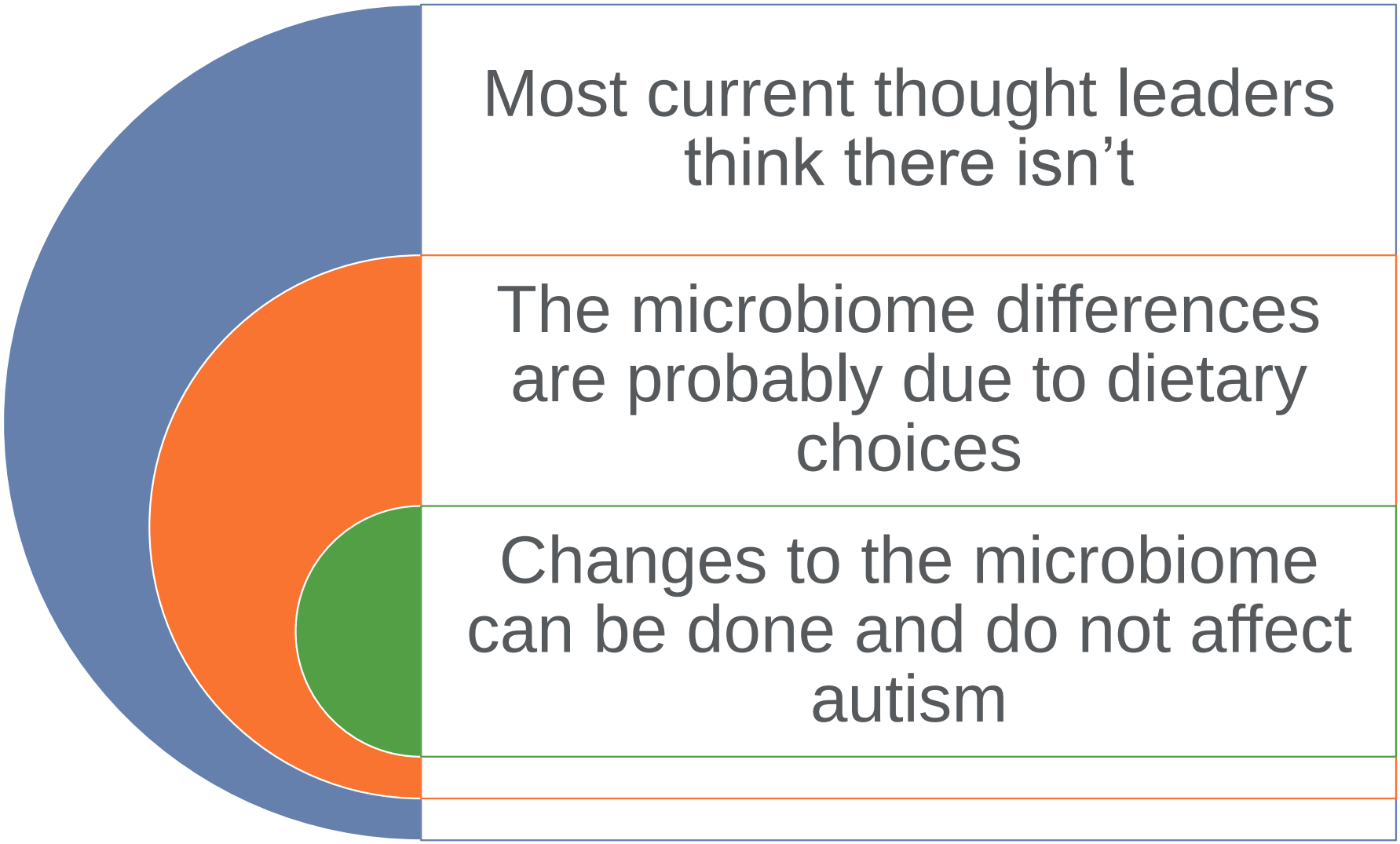
Data

- Mouse model – treat with commensal bacteria *Bacteroides fragilis*
  - Anxiety-related behaviors improved
  - Assumption in paper is that *B. fragilis* produces a polysaccharide that promotes better T-cell development and corrects imbalances
  - Question of whether certain bacteria influence early immune system development
- Evaluating stool samples
- Parracho reported greater Clostridia species in autistic group compared to unaffected siblings

Zmanian, SK et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host himmune stem. Cell. 2005. 122: 107-118

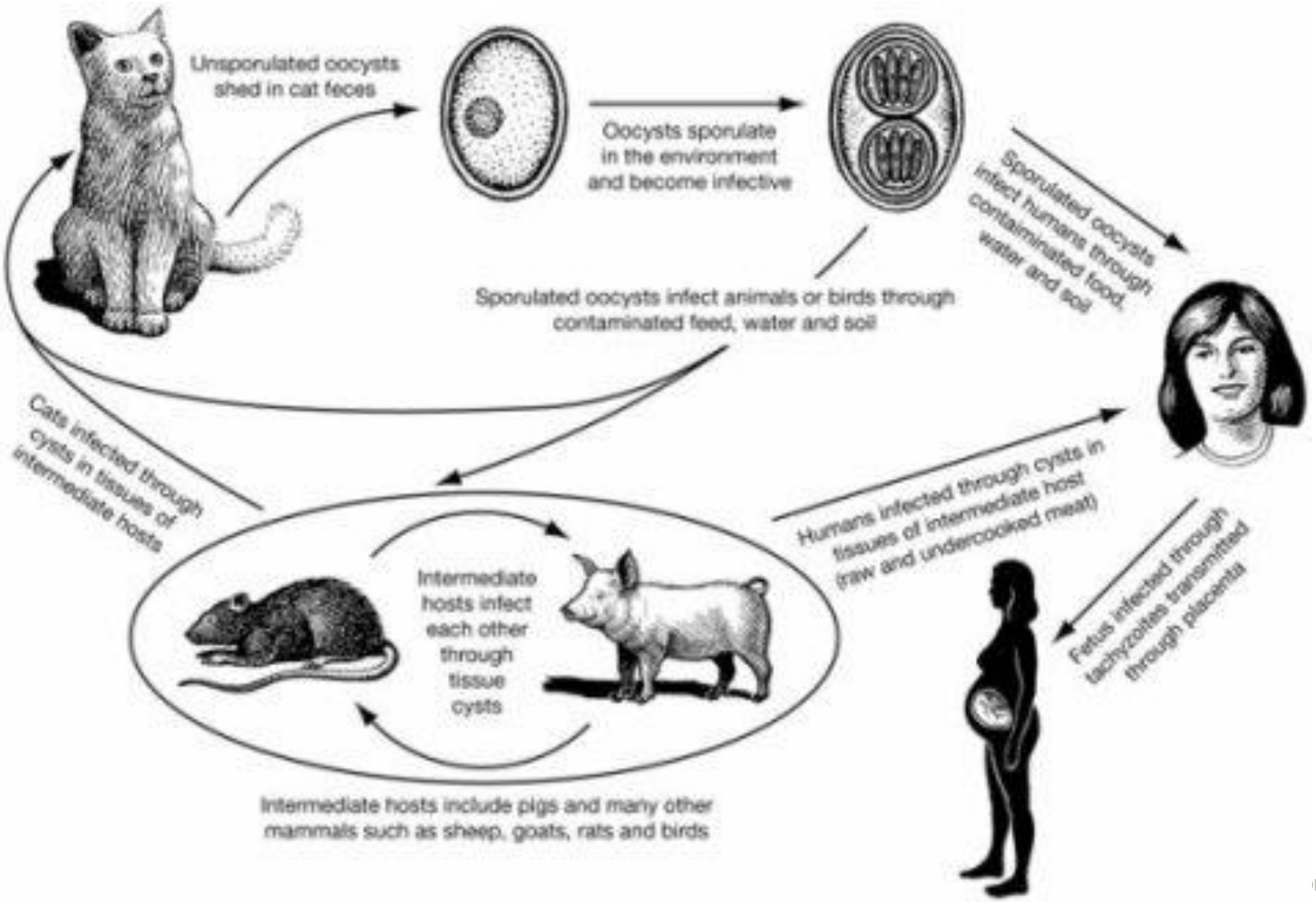
Parracho, et al. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol. 2005. 54: 987-991..

And So Is There A Connection?



Behavior?

Life Cycle of Toxoplasma gondii



Can Microbes Affect Behavior

Toxoplasma gondii in mice

- Within 3 weeks of a mouse being infected, it loses its fear of cat odor
- Researchers had a strain that could mount an immune response so should be able to be cleared from the body
- After four months, not detectable in the brain
- Mice still did not fear cat odor
- Suggests microbe had permanent change in brain

Toxoplasma gondii in humans

- Linked to increased high risk-behavior and less self-control

Skallova, et al. Decreased level of novelty seeking in blood donors infected with Toxoplasma. Neur Endocrinol Lett. 2005. 26: 480-486

Flegr et all. Correlation of dudration of latent Toxoplasma gondii infection with personality changes in women. Biol Psychol. 2000. 53:" 57-79.

Carter, CJ. Schizophrenia susceptibility genes directly implicated in the life cycle of pathogens: cytomegalovirus, influenza, herpes simplex, rubella, and Toxoplasma gondii. Schizophr Bull. 2009. 35: 1163-1182.

Low Dose Penicillin & Brain Cytokines

Experiment

- Give mice low dose penicillin in late pregnancy and early postnatal life
- Some mice received *Lactobacillus rhamnosus* JB-1

Results

- Long-term changes in gut flora
- Changes in blood brain barrier integrity
- Brain cytokines increased in frontal cortex
- Behavior
  - Anxiety-like behavior
  - Socially-impaired behavior

Results in mice with *L. rhamnosus*

- Prevents some of alterations

Leclercq, S, FM Mian, AM Stanisz, LB Bindels, E Cambier, H Ben-Amran, O Keren, P Forsythe, and J Bienenstock. Low-dose penicillin in early life indues long-term changes in murine gut microbiota, brain cytokines and behaior. Nature Communications. DOI: 10.1038/ncomms15062. 2017.

PANDAS, PANS and Acute-onset OCD: Moving Beyond the Controversy to Improved Patient Care

Susan E. Swedo, M.D.

Pediatrics & Developmental Neuroscience Branch
National Institute of Mental Health
NIH Intramural Research Program

NIMH
National Institute of Mental Health

PDN

PANDAS Comorbid Symptomatology

Sleep disorders 80%

Insomnia, night terrors, refusal to sleep alone

Behavioral regression

Separation anxiety (98%), baby talk, tantrums

Inability to concentrate 90%

Hyperactivity, inattentiveness70%

Aggressiveness 60%

Learning difficulties 60%

Eating disorder 20%

Hallucinations 10%

Terror stricken look (mydriasis) or Hyper-alert appearance 80%

Urinary frequency, urgency, enuresis (night & daytime) 90%

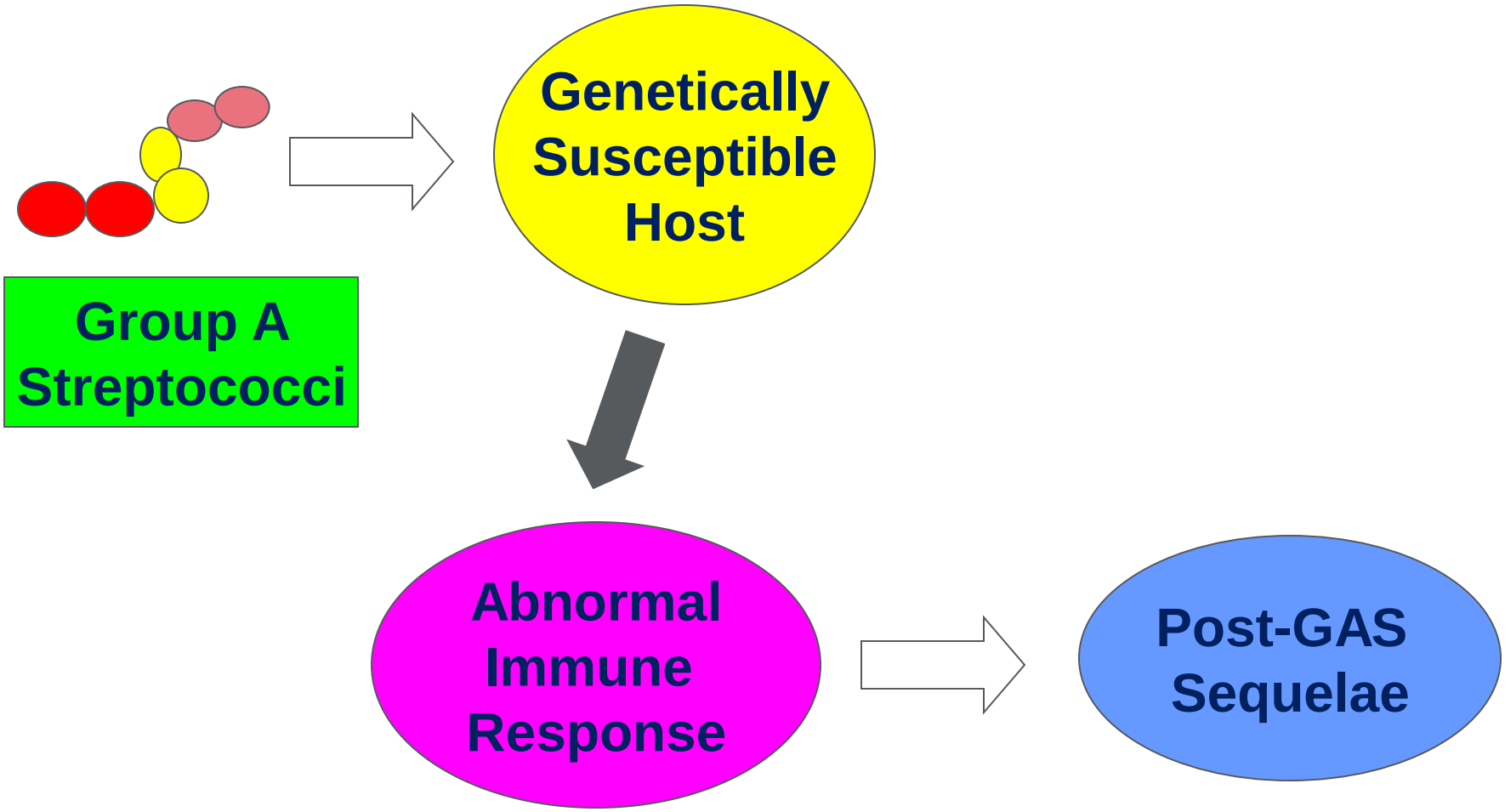
Handwriting deterioration 90%

Tics 70%

Short-term memory loss 60%

Sensory hypersensitivity or insensitivity 40%

PANDAS Model of Etiopathogenesis



GAS Infections Correlate with Abnormal Movements and Hyperactivity

Tanya Murphy and colleagues at Univ FL

In-person observations among 693 elementary school children revealed:

- Direct correlation between + GAS throat cultures and
- Presence of tics, adventitious movements and problem behaviors
- Recurrence of GAS infections increased the risk.

TK Murphy et al, Biol Psychiatry 2007

"Prospective Identification and Treatment of Children with PANDAS" *Murphy & Pichichero*

12 patients identified over 3 years period

7 boys & 5 girls presented with neuropsychiatric symptoms related to GABHS infections

- 100% with OCD (3/4's were germ-related) and emotional lability
- 58% (7/12) with urinary frequency or enuresis
- 42% (5/12) with acute separation anxiety
- 33% (4/12) with tics or handwriting changes

Antibiotic treatment of GABHS infections reduced symptom severity in 5 – 21 days

Arch Ped Adolesc Med 2002;156:356-361

The Microbiome Mutiny Hypothesis

Mutiny from the Microbes

Hypothesis

- Microbes may make coordinated change to virulence factors to leave an older host or seriously ill

Reason

- Increase ability to jump to other hosts

Is there data?

- Increased diarrhea in elderly
- Higher pneumonia and urinary tract rates
- Increased reactivation of things like herpesviruses

Microbes would need data about their host's health

Rozsa, Ljos, Peter Apari, and Victor Muller. The microbiome mutiny hypothesis: can our microbiome turn against us when we are old or seriously ill? Biology Direct. DOI 10.1186/s13062-014-0034-5

Non Human Microbiome Issue

Antibiotic Exposure and Bees

Issue

- In apiculture, antibiotics are often used to prevent bacterial infections in larval bees.

Concern

- Microbial imbalance as antibiotics alter the honeybee gut composition

Experiments

- Antibiotics did lead to decreased survivorship in both the hive and lab experiments when then exposed to opportunistic bacterial pathogens

Raymann, K, Z Shaffer, N Moran. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honebees.

Side Benefits

Probiotics

Fermented milk can be seen back in Egyptian hieroglyphics

1800’s – scientists started looking at benefits of fermented milk products

1930’s – yogurts became fashionable probiotic

Studies going on now

- Antibiotic-associated diarrhea
- Irritable bowel syndrome
- Pediatric diarrhea
- Treating *C. difficile*
- Constipation
- Treating *H. pylori*
- Allergies

Hygiene

Listerine

- Started as floor cleaner
- Invented the word halitosis

Bathing

- Used to do it once a week
- Bathing/showering every day was massive ad campaign by soap companies to sell more soap
- Over showering dry out skin – remove protective lipids, just under arms or groin is fine

Replacing Showering?

AOBiome sells mist that contains Nitrosomonas eutropha

- Ammonia-oxidizing bacteria
- Hypothesis is that it used to live freely on us and continual washing, deodorant, etc. has taken it away

Is it effective?

- Inventor is an MIT chemical engineer who hasn't showered for 12 years
- Chairman uses soap once or twice a month and shampoos 3x per year

L'Oréal, Estée Lauder, Clinique looking at & patenting probiotics for skin

TEST
TARGET
TREAT™

Diagnostics Can Help With Therapeutic Decisions

Advantages of Rapid Testing for Infectious Diseases

Faster directed therapy to reduce:

- **antibiotic resistance**
- **hospital length-of-stay**

Less adverse consequences

Teachable moment

Reduced length-of-stay in Emergency Department

Timely application of **appropriate infection control** procedures

Global Antibiotic Resistance Crisis

“There aren’t enough good rapid tests to confirm the professional judgment of the doctor,.. this is not acceptable: we need to encourage more innovation and ensure that useful products are used. I call on the governments of the richest countries to mandate now that by 2020, all antibiotic prescriptions will need to be informed by a rapid diagnostic test wherever one exists.[12] ”

- Jim O’Neill 2016

12. O’Neill, J. Tackling drug-resistant infections globally: Final report and recommendations. The Review on antimicrobial resistance. May 2016.

What's driving the need for rapid accurate diagnostic tests?

Transition to "patient-centered" value based health service delivery[8]

- Get the diagnosis right the first time
- Diagnose in an actionable timeframe
- Early optimal treatment selection

- Avoid the waste of unnecessary investigations
- Avoid the waste of over treating
- Avoid the consequences of incorrect patient management

- Better health outcomes and reduced healthcare costs

The results of diagnostic tests are immensely influential,
affecting around 60–70% of all clinical decisions,
although they still amount for only 4–5 % of healthcare costs.[8]

8. Akhmetov, I. and Bubnov, R.V. Assessing the value of innovative molecular diagnostic tests in the concept of predictive, preventive, and personalized medicine. *The EPMNA Journal* (2015) 6:19.

TEST
TARGET
TREAT™

Question:

What is the future of the microbiology laboratory?

Conclusions

Antibiotic resistance is an immediate global threat

Treating empirically can lead to increased resistance

Directed therapy after diagnosis can reduce antibiotic resistance while improving care and reducing costs

Discussion