The good, the bad and the ugly

Our microbiome and it’s effect on health and disease.

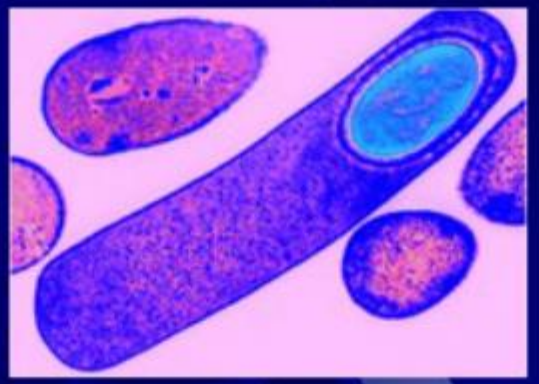
Objectives

- Discuss C difficile infection including treatment options
- Explain process of Fecal MicrobiotaTransplantation
- Discuss the microbiome and how it affects health and disease
- Discuss the potential benefits of probiotics

Clostridium difficile

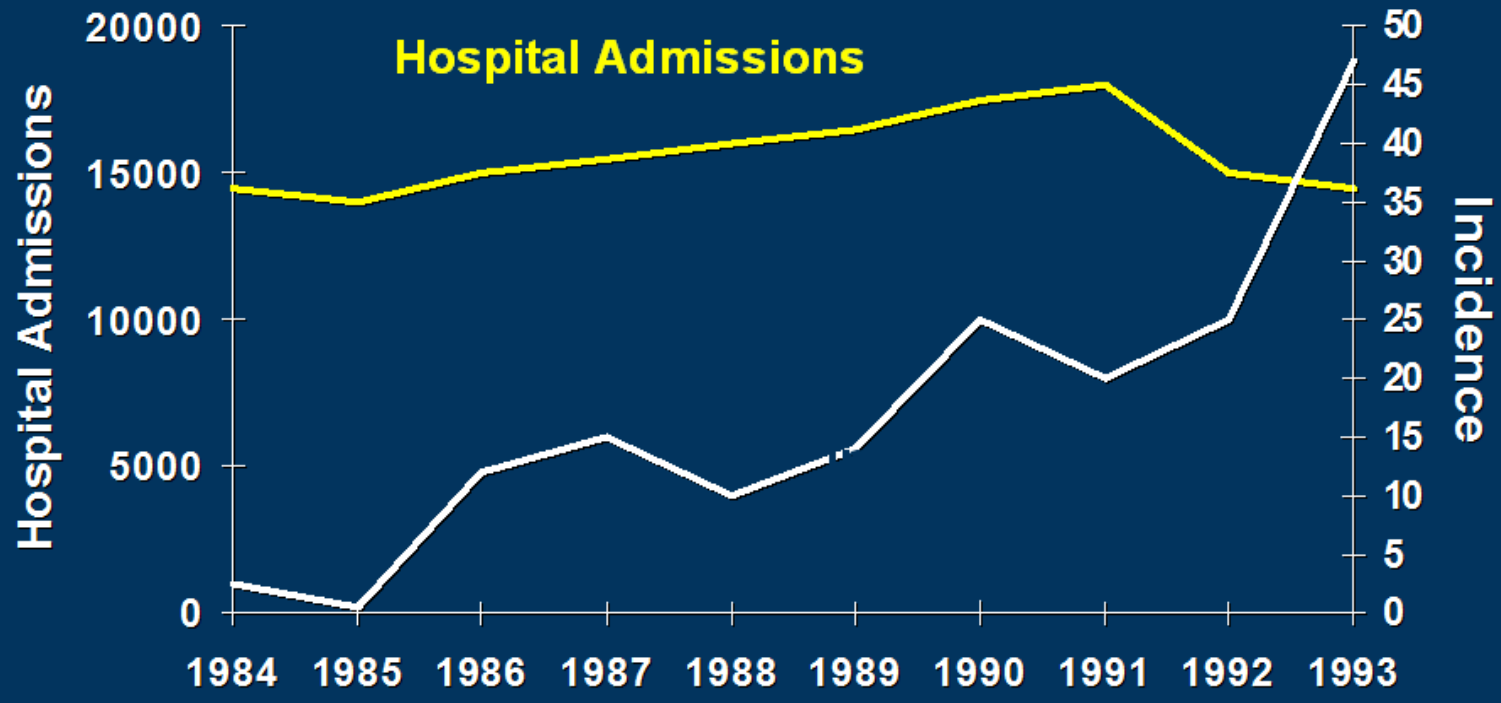
- Discovered in 1935 by Hall & O'Toole.
- Motile anaerobic gram-positive bacillus with oval, sub-terminal spores
- Named "difficult clostridium" due to its resistance in isolation and growth.
- In 1978 C. difficile produced toxin was found in patients with antibiotic-associated pseudomembranous colitis.
- Today, known as the major cause of diarrhea and colitis in patients exposed to antibiotics.
- Community-acquired C. Difficile detected in U.K. In outpatients not exposed to antibiotics within 3 months.

Clostridium difficile



Causes 400,000 cases of diarrhea annually. A new mutation produces 20 times the toxin of the old version. In an outbreak in Quebec, it infected 1,703 patients; 33 had to have colectomy, 117 people died.

Rising Incidence of C.difficile



- Incidence of C.difficile by year

Adapted from Jobe BA et al. *Am J Surg.* 1995;169:480-483.

Pathogenesis of CDAD

Antibiotic therapy

↓

Alteration in colonic microflora

↓

C. difficile exposure and colonization

↓

Release of toxin A and Toxin B

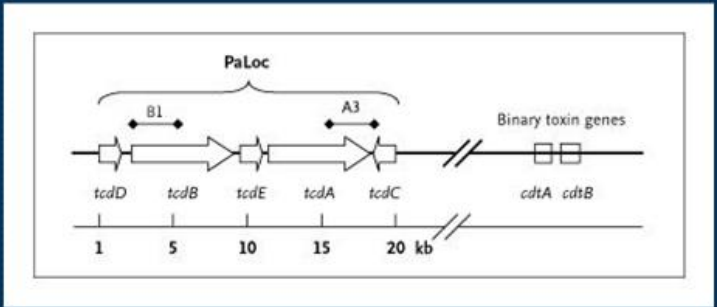
↓

Colonic mucosal injury and inflammation

- Adapted from Kelly CP et al Ann Rev Med 1998;48:375-390
- 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

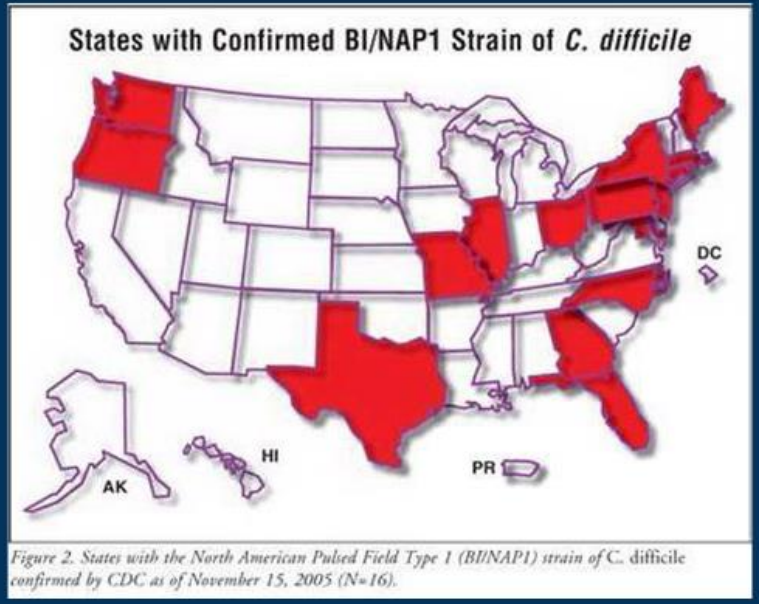
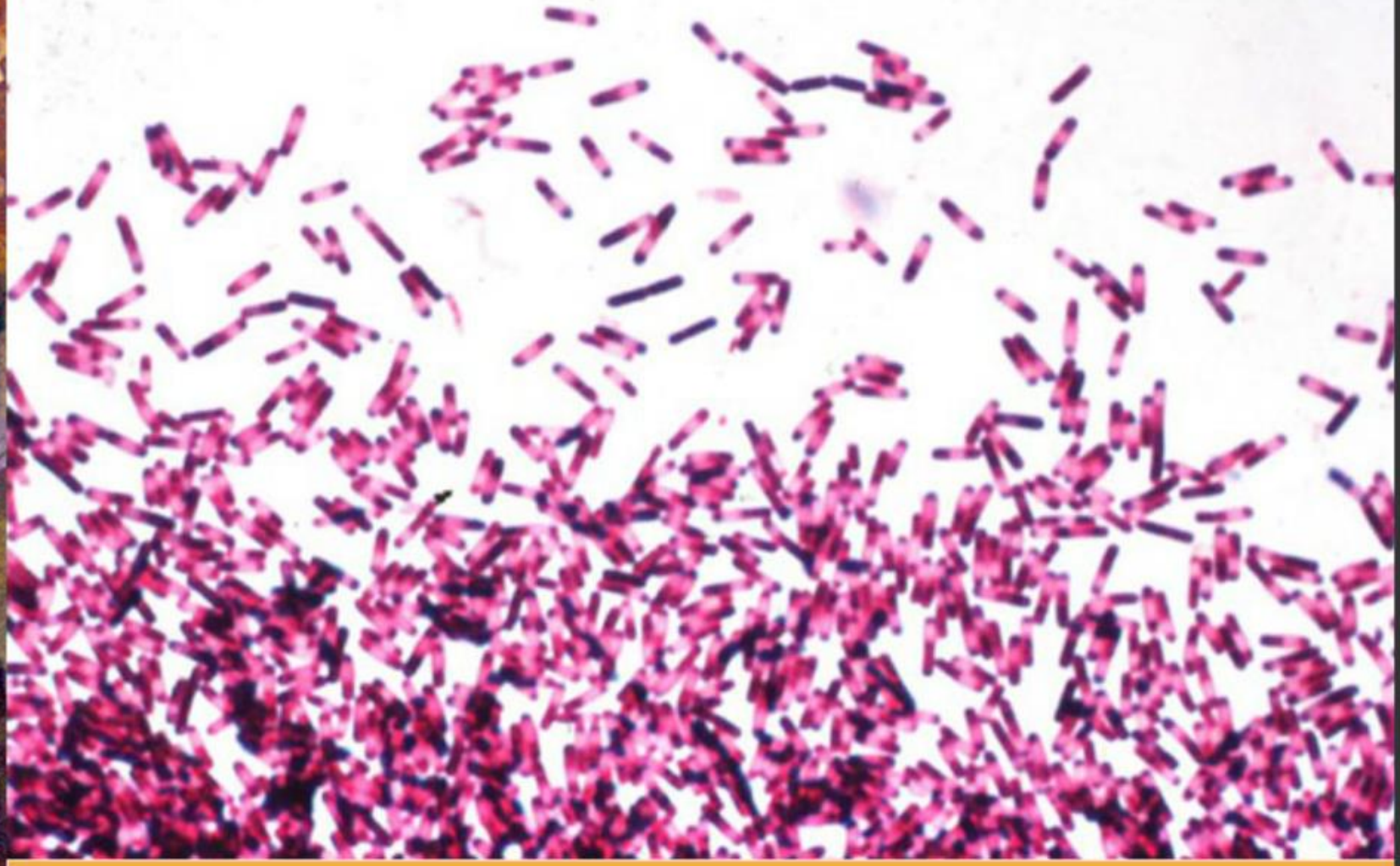


Figure 2. States with the North American Pulsed Field Type 1 (BI/NAP1) strain of C. difficile confirmed by CDC as of November 15, 2005 (N=16).

- **McDonald NEJM 2005**

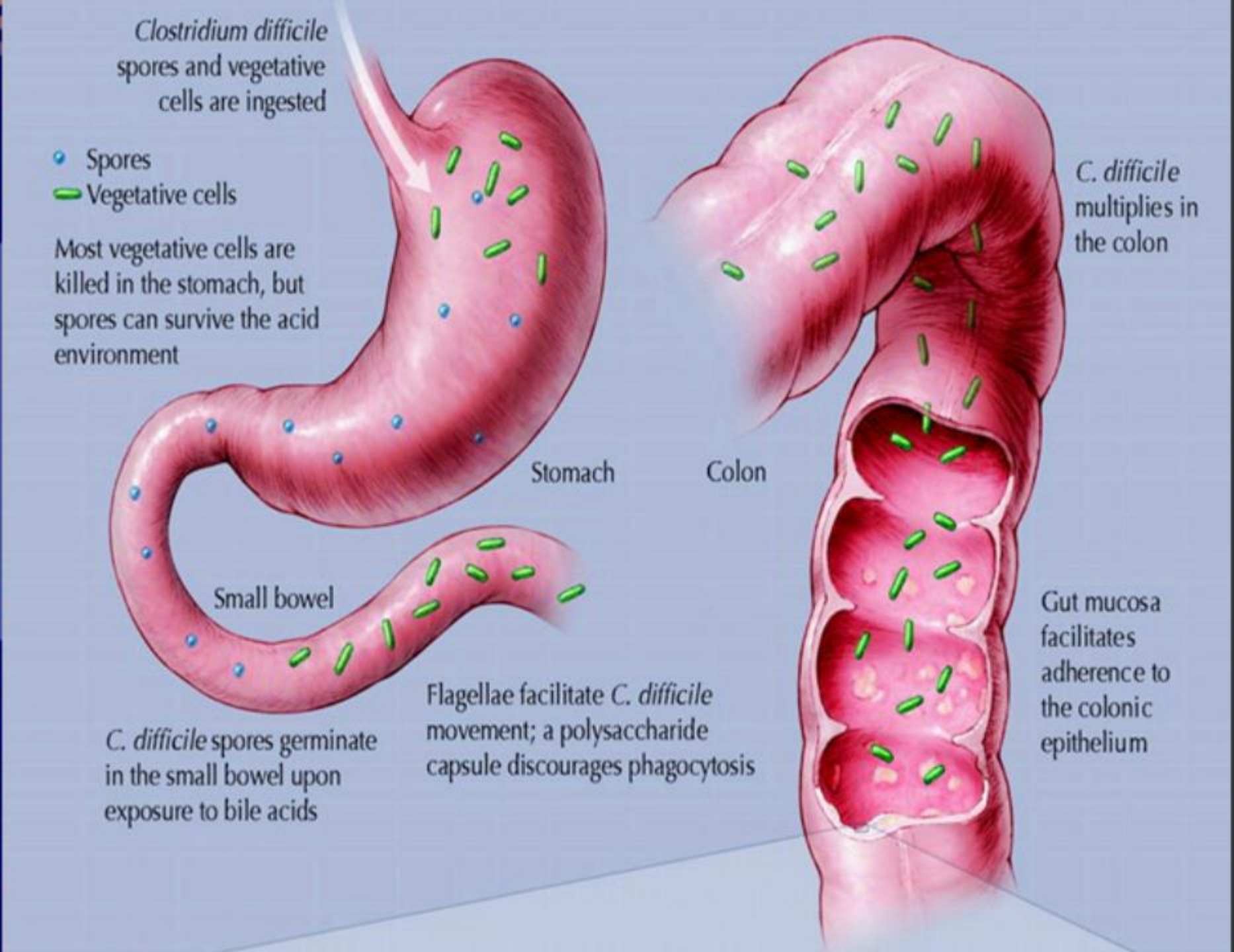


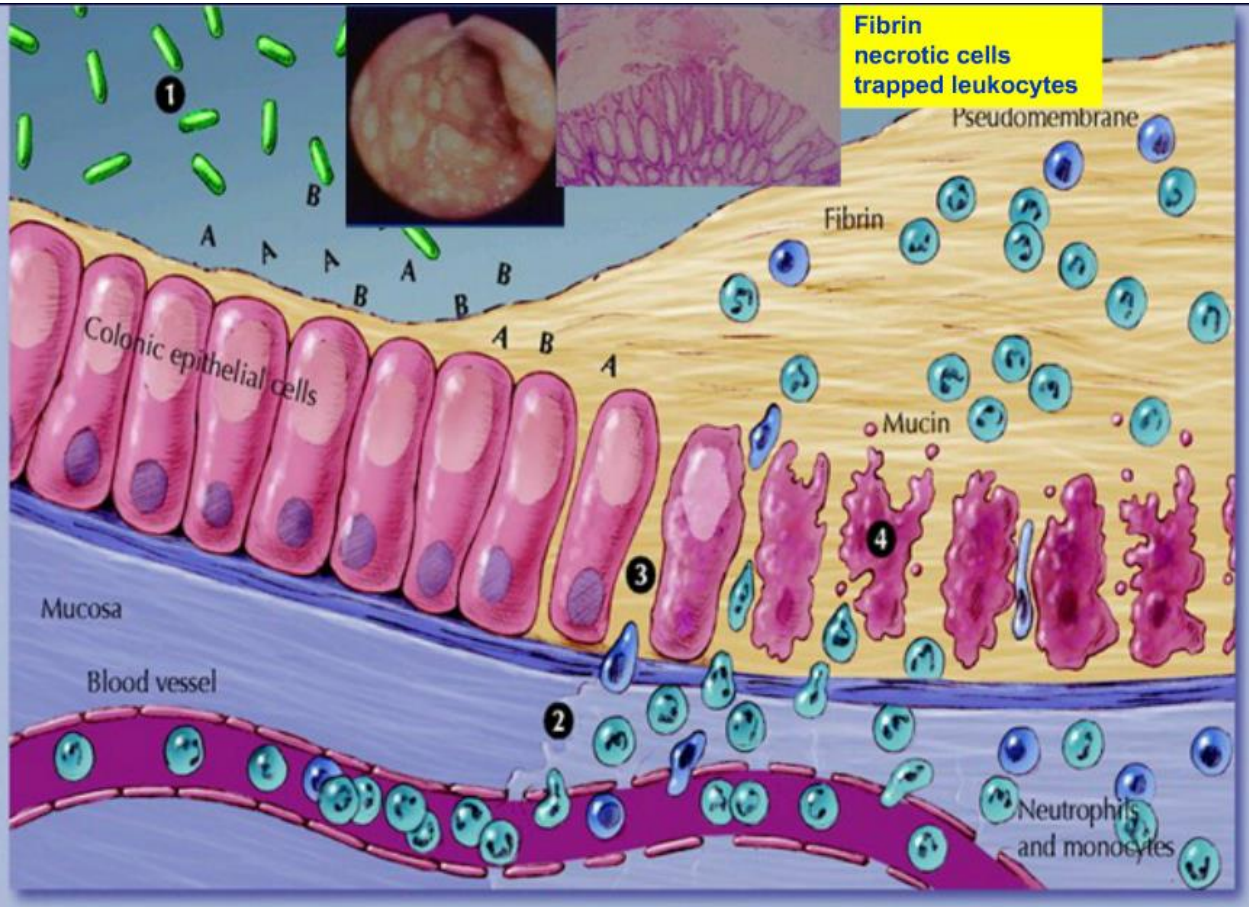
Clostridium difficile

Clinical manifestations

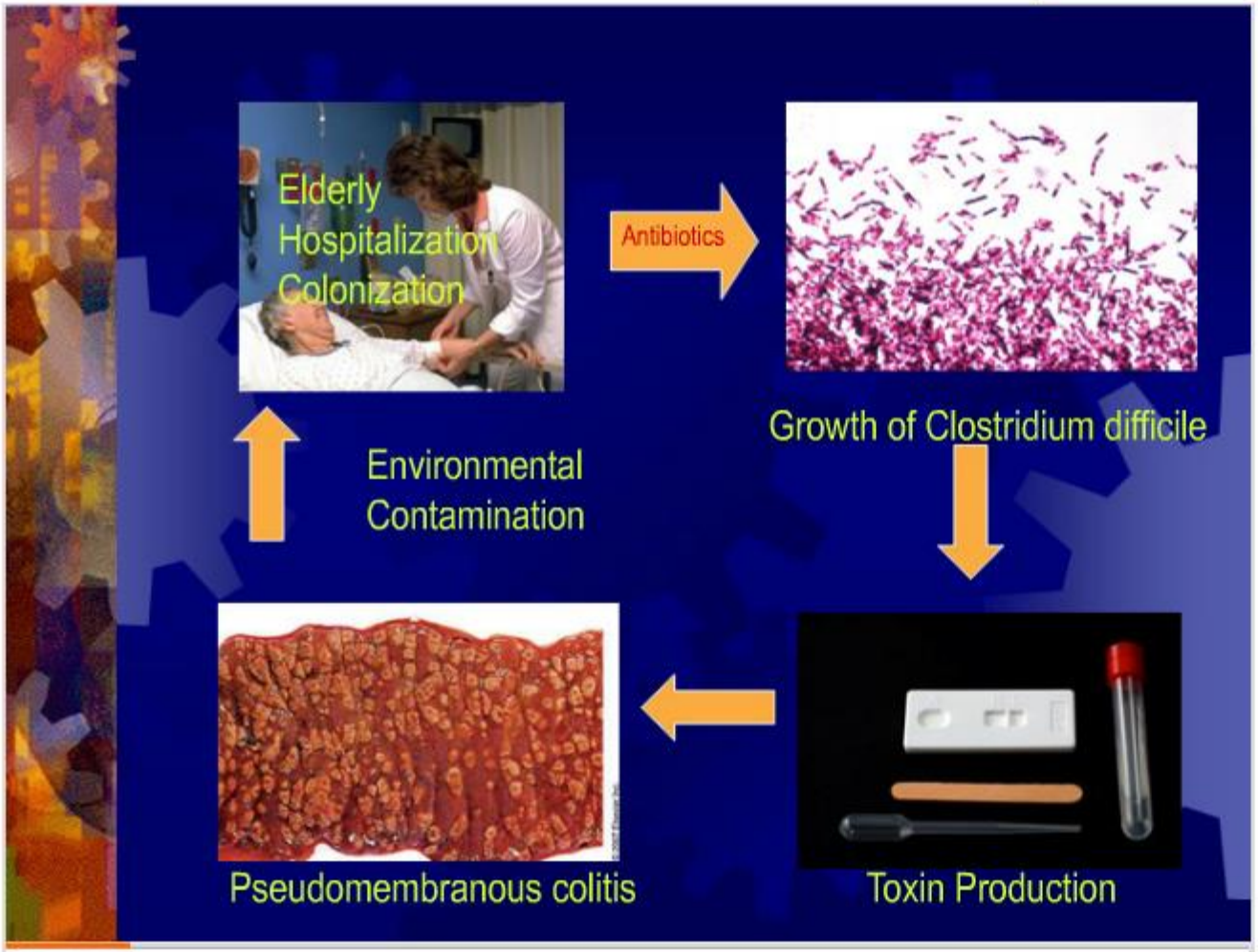
Type of infection	Diarrhea	Other symptoms	Physical Examination
Asymptomatic carrier	Absent	Absent	Normal
AB-associated diarrhea without colitis	Mild to moderate	Crampy lower abd discomfort	Slight lower abdominal tenderness
AB-associated colitis without pseudo-membranes	10+ loose bm/day; fecal leukocytes;occult blood	Nausea, anorexia fever, malaise, dehydration, leukocytosis	Abdominal distention, tenderness
Pseudo- membranous colitis	>10 loose bm/day, fecal leukocytes, occult blood	Nausea, anorexia fever, malaise, dehydration, electrolyte imbalance, leukocytosis	Marked abdominal tenderness, distention

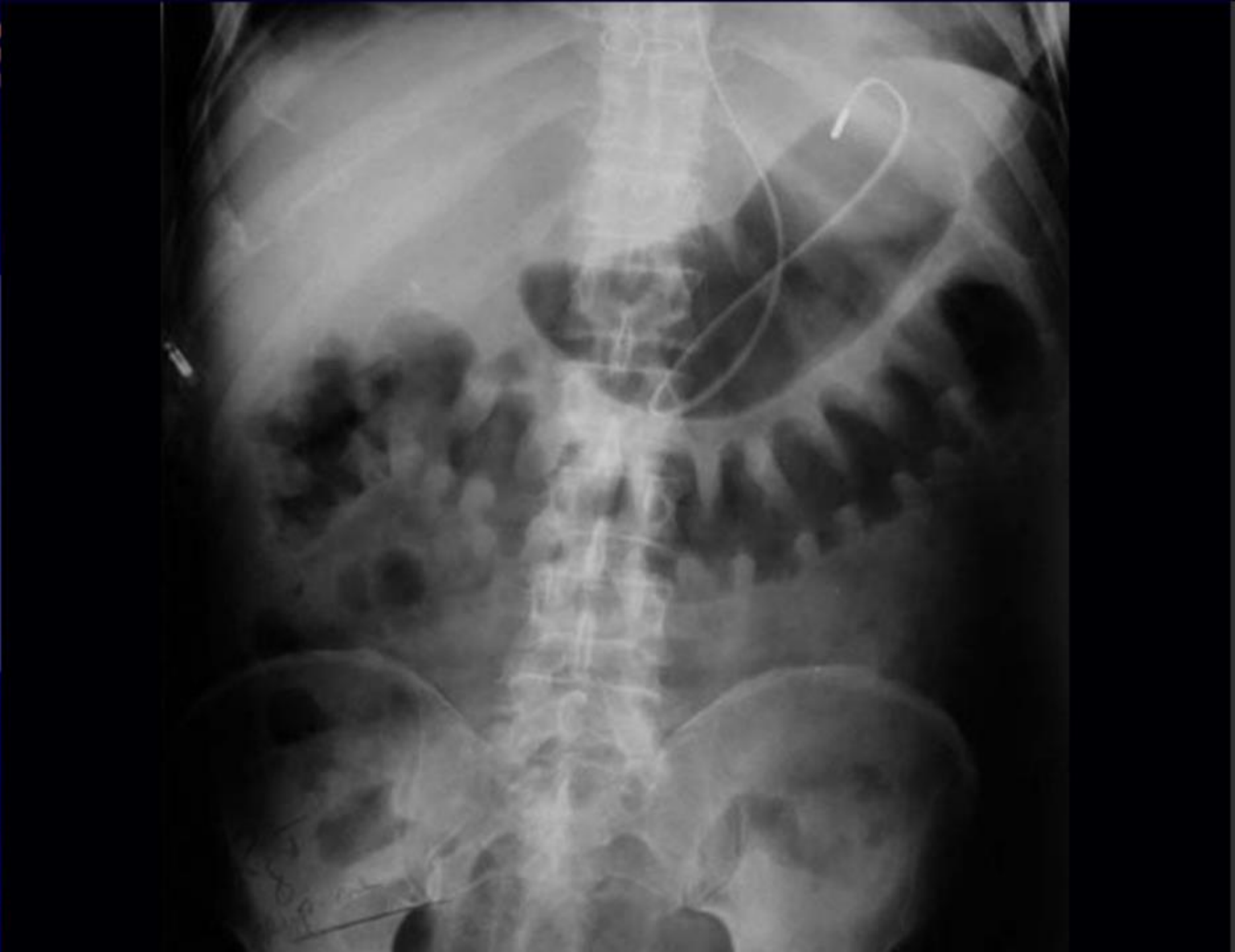
Type of infection	Diarrhea	Other symptoms	Physical Examination
Fulminant colitis	May be severe or decreased due to colonic dilation (toxic megacolon) &/or paralytic ileus	Lethargy, high fever, chills, tachycardia, abdominal pain, hypotension, dehydration, marked leukocytosis, electrolyte imbalance	Severe lower or diffuse abdominal tenderness, distention

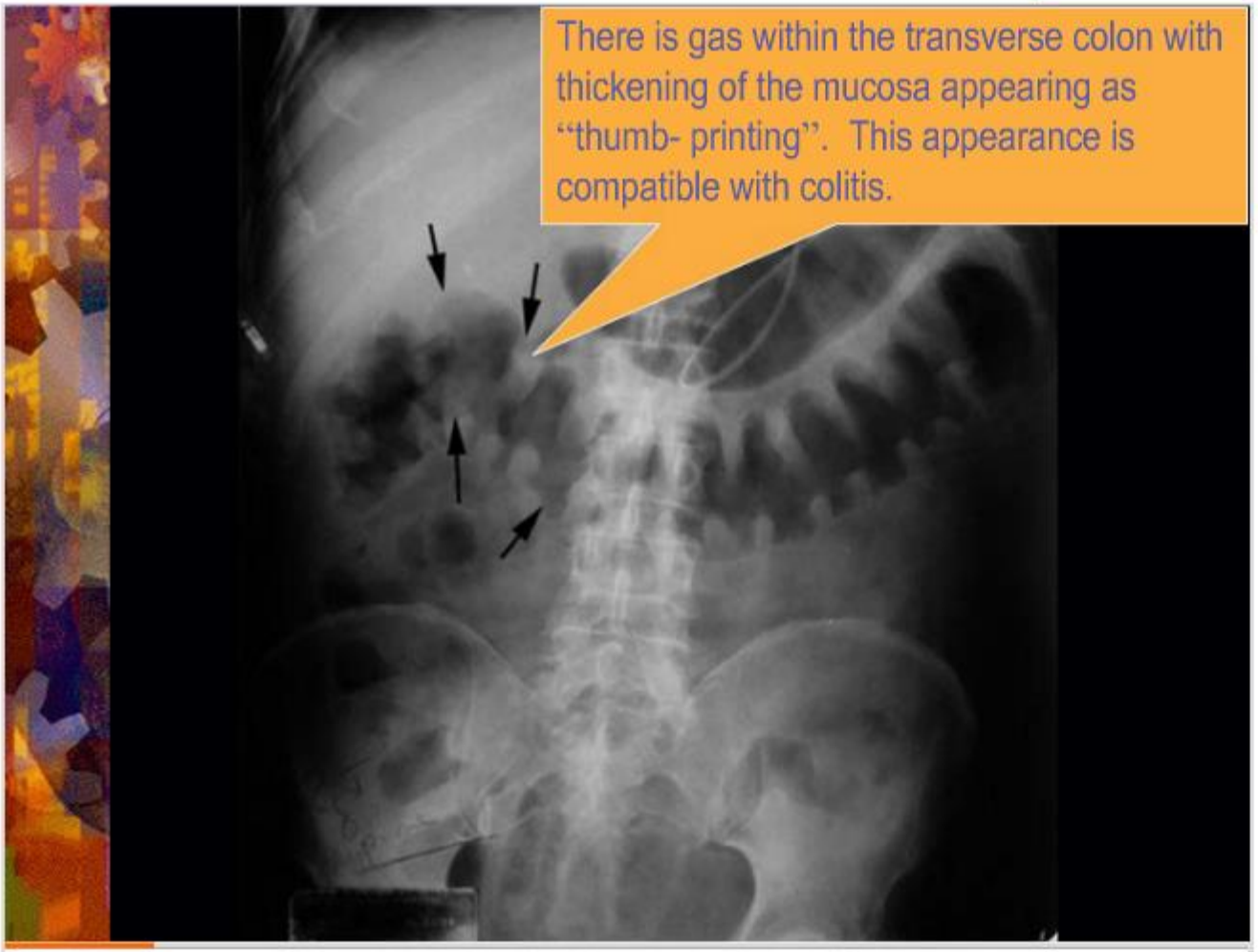

Clostridium difficile spores and vegetative cells are ingested
Spores
Vegetative cells
Most vegetative cells are killed in the stomach, but spores can survive the acid environment
Stomach
Small bowel
C. difficile spores germinate in the small bowel upon exposure to bile acids
Flagellae facilitate *C. difficile* movement; a polysaccharide capsule discourages phagocytosis
Colon
C. difficile multiplies in the colon
Gut mucosa facilitates adherence to the colonic epithelium

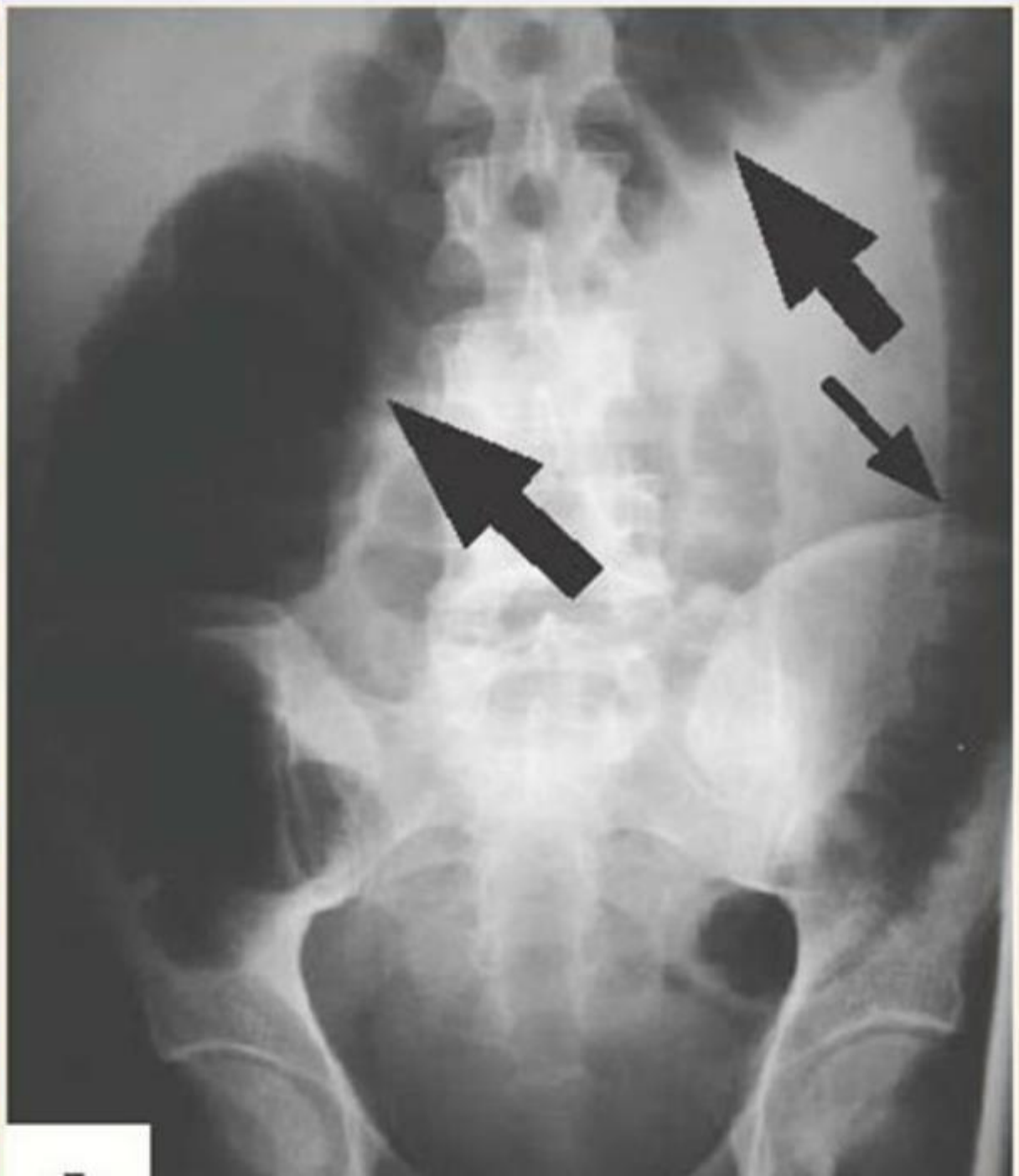


C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (**1**). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (**2**), opening of epithelial cell junctions (**3**) and epithelial cell apoptosis (**4**). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (**5**) and watery diarrhea.

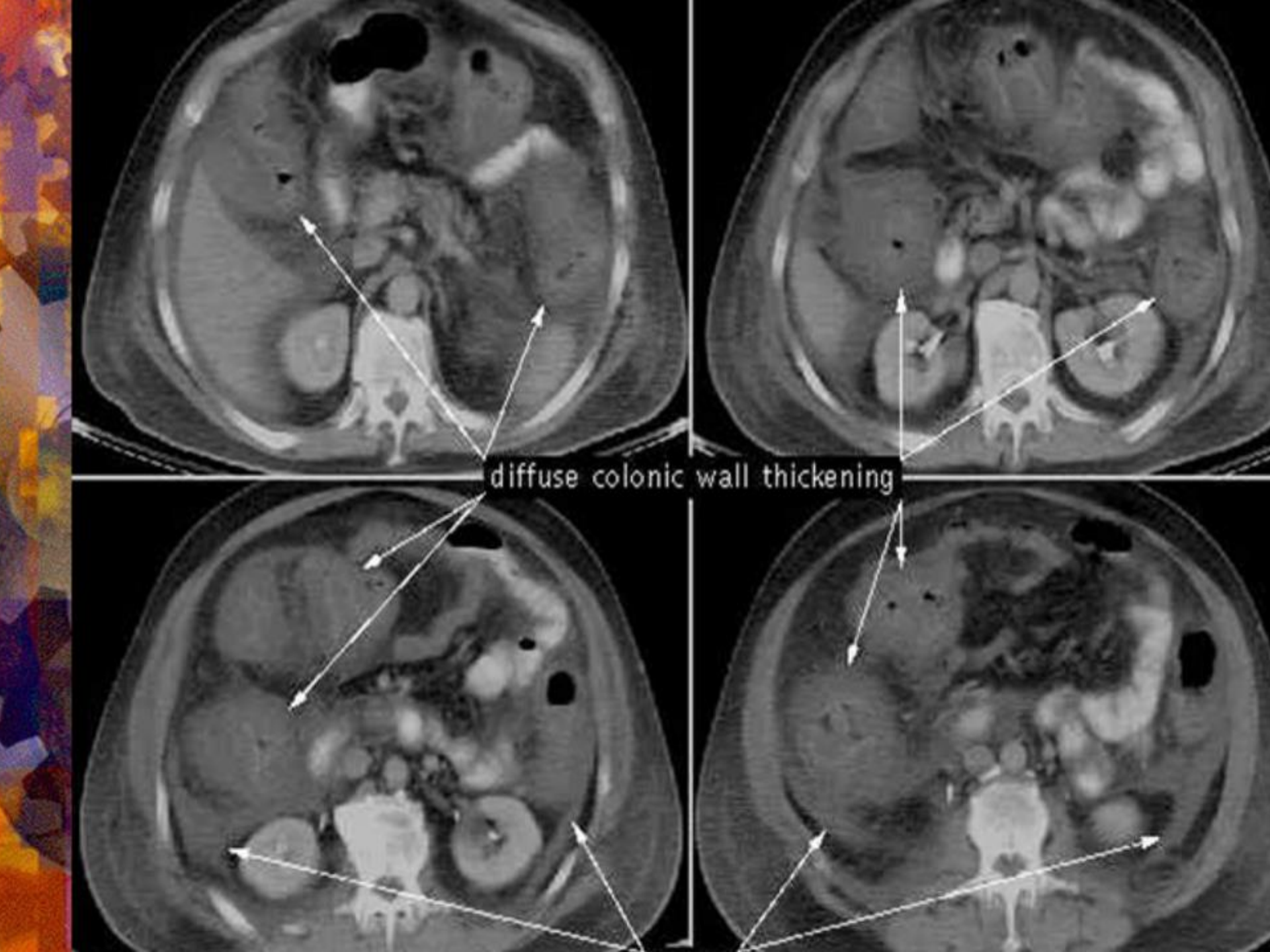

Elderly
Hospitalization
Colonization
Antibiotics
Growth of Clostridium difficile
Environmental
Contamination
Pseudomembranous colitis
Toxin Production

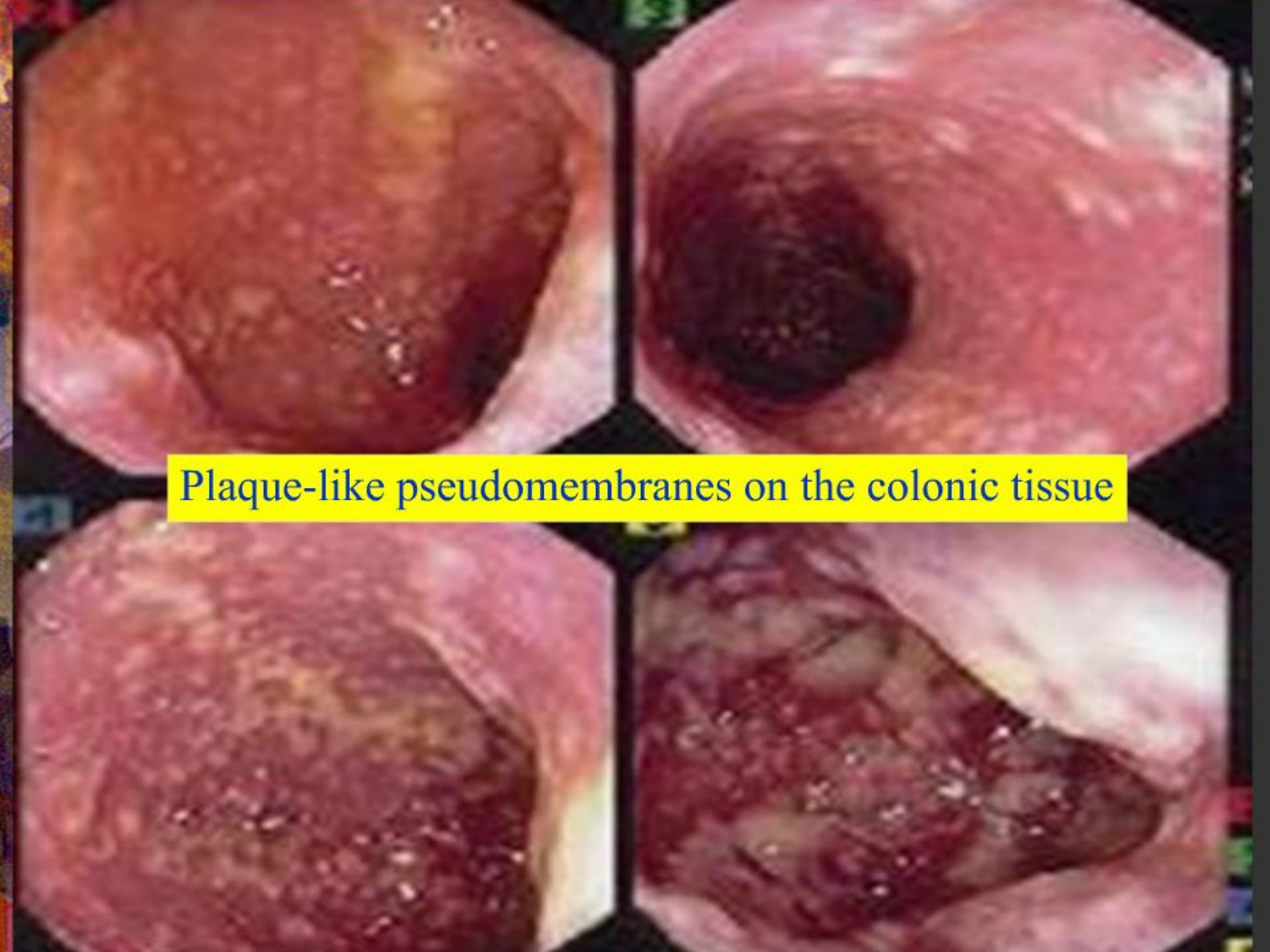




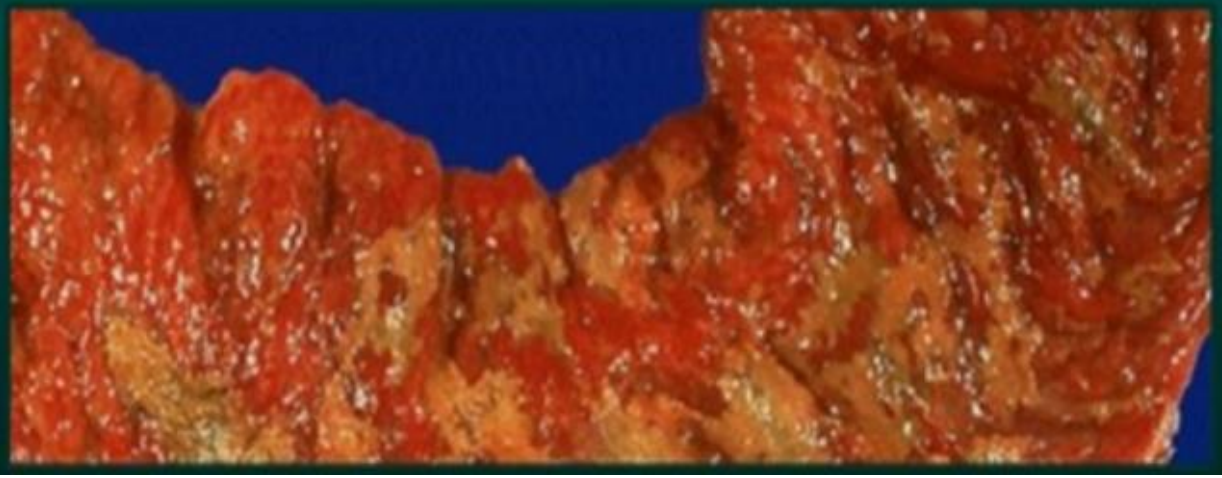


Features of *C difficile* colitis: *A) Plain film of abdomen showing* ***bowel wall thickening, loss of haustral markings*** *(thin arrow) and* ***dilation*** *of the ascending and transverse colon (thick arrow);*

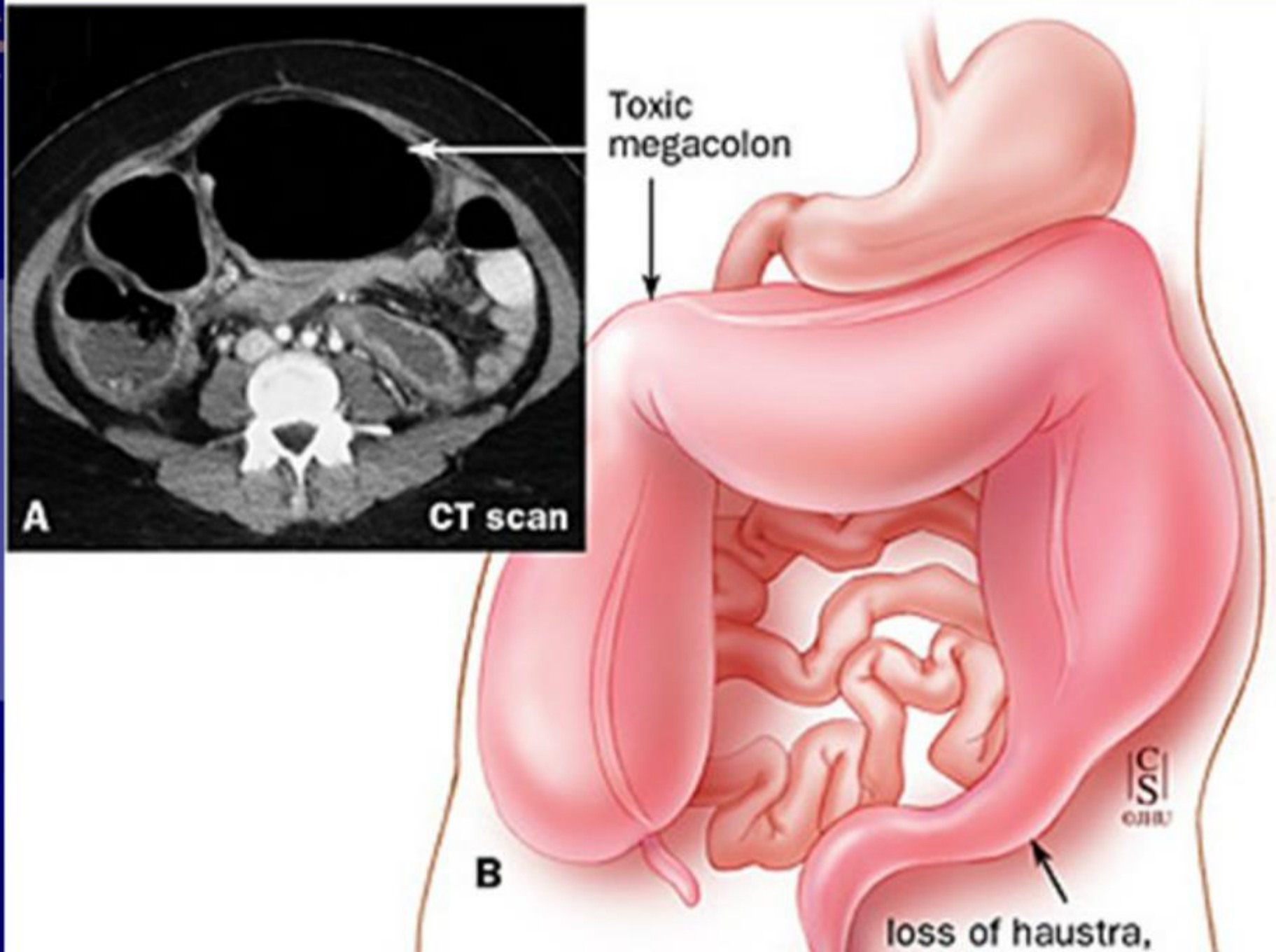

diffuse colonic wall thickening


Plaque-like pseudomembranes on the colonic tissue

Pseudomembranous Ulcerative Colitis



C. difficile overgrowth


A
CT scan
Toxic
megacolon
B
loss of haustra,

Toxic megacolon is defined as a severe episode of colitis with segmental or total dilatation of the colon. The mortality rate is high at 20%.

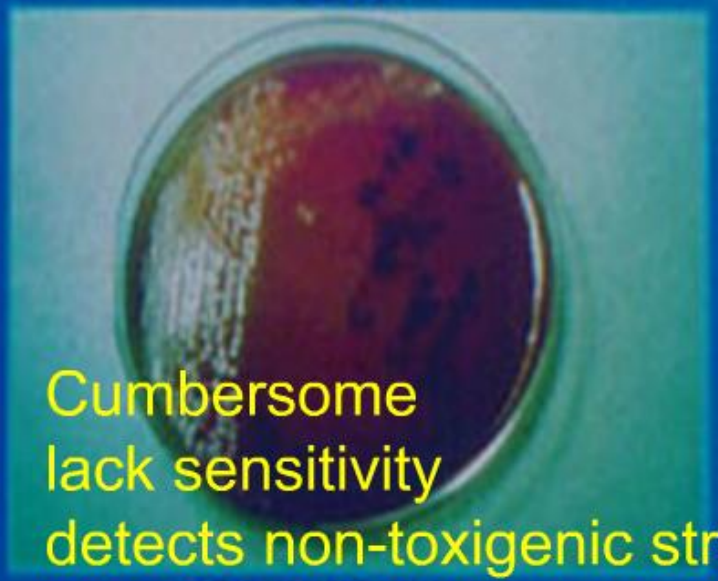
Pathologically, acute fulminant colitis is associated with neuromuscular degeneration and a rapid and extensive colonic dilatation.

C. DIFFICILE

Toxin assay

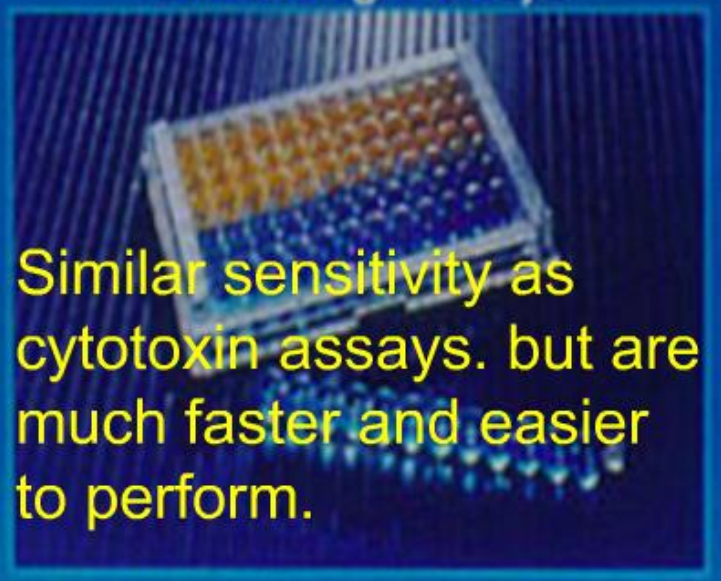
Cytopathic Rounding neutralized with Clostridium sordelli Antitoxin Specific but low sensitivity

Culture



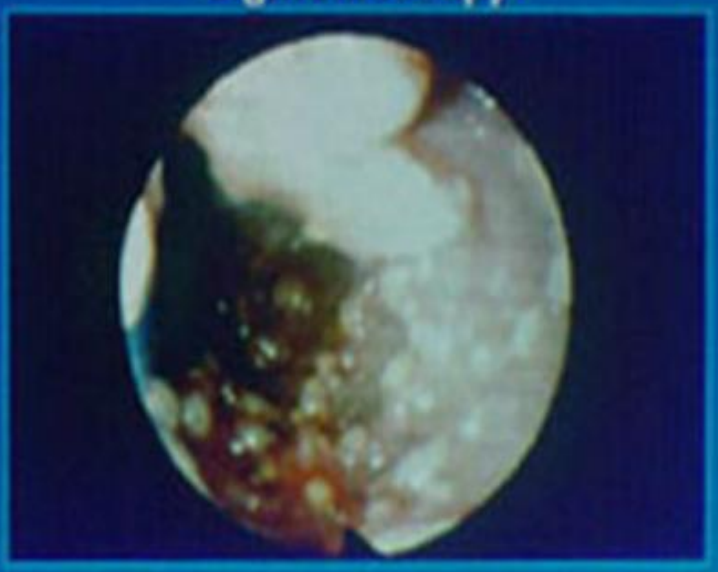
Cumbersome
lack sensitivity
detects non-toxigenic strain

Immunologic assays



Similar sensitivity as cytotoxin assays. but are much faster and easier to perform.

Sigmoidoscopy



Enzyme-linked immunoassay for toxin.

- These rapid tests use monoclonal antibodies to detect toxin, and are probably the most widely used assays. Commercial kits are available that detect toxin A, toxin B, or both toxins.27.
- These tests have lower **sensitivity (63%-99%)** than cytotoxin culture, but high **specificity (85%-100%)**. If test results are reported negative, sending one or two additional stools on subsequent days could improve sensitivity.
- In addition, if toxin A alone is tested, diarrhea secondary to toxin A negative toxin B positive strains will be missed.

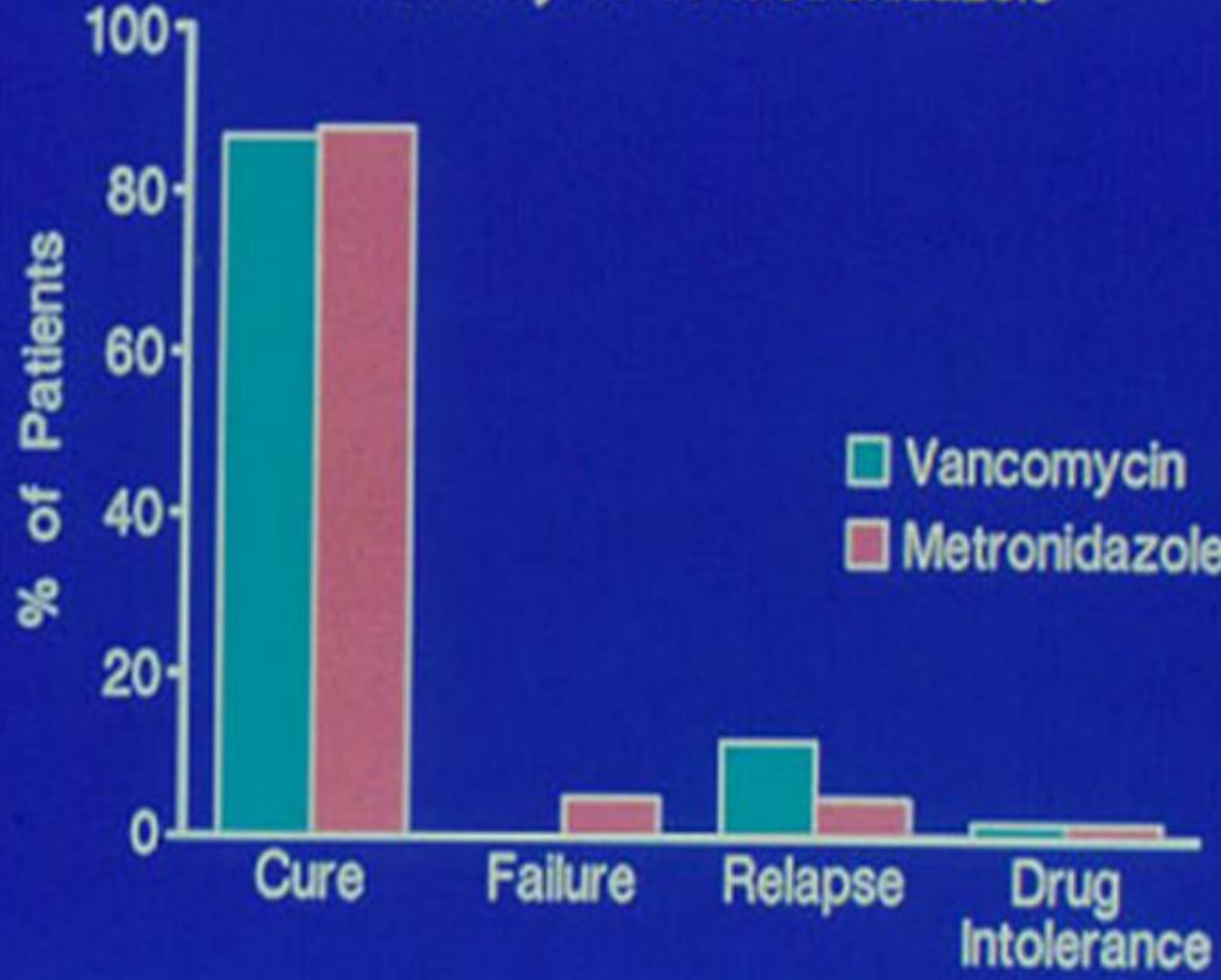
Up to 50% of neonates have colonizing *C. difficile* in their intestines but an inherent protection against toxin A. It is found in the feces of only 3% of normal adults.

Infection by *C. difficile* is a very important nosocomial pathogen may result in an asymptomatic carrier state, mild diarrhea, or at the extreme end of the spectrum, pseudomembranous colitis, a severe colitis which can lead to megacolon, sepsis, and death.

C. DIFFICILE

Antibiotic-associated Colitis: Treatment

Vancomycin vs Metronidazole



Recommendations for *Clostridium difficile*–Associated Diarrhea

[Bartlett: Ann Intern Med, Volume 145(10).November 21, 2006.758-764]

- In 1978 *Clostridium difficile* became the established pathogen in the vast majority of cases of **antibiotic-associated diarrhea** and nearly exclusive cause of **pseudomembranous colitis**.
- Risks for infection include **hospitalization**, **advanced age**, gastrointestinal surgery or gastrointestinal procedures, and **antibiotic exposure**.
- There is a new epidemic of heavy toxin producing *C. difficile* **(BI, NAP1, or ribotype 027)** infection that is occurring more frequently and is more serious and more refractory to therapy. Evidence of the severity of the infection includes high rates of **toxic megacolon**, **leukemoid reactions**, **severe hypoalbuminemia**, **requirement for colectomy**, **shock**, **and death**.
- Successful management of patients with this strain requires **early detection of infection**, **rapid treatment**, and implementation of **infection control**, sometimes including **antibiotic control**.

Of the patients who developed Clostridium difficile asscociated diarrhoea, **30% had no previous use of antibiotics**. ===□ Nosocomial Infection

Clostridium difficile – An emerging epidemic in European Health Care? Background paper available for public consultation.

Clostridium difficile–Associated Diarrhea

- Clindamycin followed by ampicillin or amoxicillin played prominent roles in the 1970s, but these were largely supplanted by cephalosporins in the 1980s.
- The rate per 100 000 persons older than 65 years of age was 20 times higher than that in persons younger than 20 years of age. [**Karlström O, Fryklund B, Tullus K, Burman LG.** A prospective nationwide study of *Clostridium difficile*-associated diarrhea in Sweden. The Swedish *C. difficile* Study Group. Clin Infect Dis. 1998;26:141-5.]
- 20% to 40% rate of colonization in hospitalized adults compared with 2% to 3% in healthy adults. [**McFarland LV, Mulligan ME, Kwok RY, Stamm WE.** Nosocomial acquisition of *Clostridium difficile* infection. N Engl J Med. 1989;320:204-10.]

C. difficile – Antibiotic Risk

High Risk Antibiotics:

Cefotaxime
Ceftriaxone
Cefalexin
Cefuroxime
Ceftazidime
Ciprofloxacin
Moxifloxacin
Clindamycin (low dose)

Medium Risk Antibiotics:

Meropenem
Ertapenem
Clindamycin (high dose)
Co-amoxiclav
Tazocin
Erythromycin
Clarithromycin

C. difficile – Antibiotic Risk

Low Risk Antibiotics:

Benzyl penicillin
Amoxicillin
Flucloxacillin
Tetracyclines
Trimethoprim
Nitrofurantoin
Fusidic acid
Rifampicin

Gentamicin
Metronidazole
Vancomycin
Teicoplanin
Synercid
Linezolid
Tigecycline
Daptomycin

The Rising Incident of **Community Acquired Clostridium Difficile Infection** in U.K.

[Dial S, et al. Use of gastric acid-suppressive agents and the risk of community-acquired *Clostridium difficile*-associated disease. *JAMA* 2005;294(23):2989–95.]

- A recent report from the UK indicated that the incidence of *C. difficile* in patients diagnosed by their general practitioners had increased from fewer than one case per 100,000 in 1994 to 22 per 100,000 in 2004.
- 63% of case patients had ***not*** received any antibiotics within the three months prior to diagnosis
- The use of **proton pump inhibitor gastric acid suppressants** (such as esomeprazole [Nexium]) increased patients' risk of acquiring *C. difficile* disease.

Soap and water for handwashing rather than alcohol-based hand hygiene.

Alcohol does not kill spores, so people should wash with **soap and water (to remove spores mechanically)** instead of using alcohol-based hand sanitizers when *C. difficile* is known or suspected.

Attempts to prevent infection with prophylactic metronidazole or oral vancomycin may actually increase the rate of *C. difficile* carriage.

The major complication of antibiotic treatment of *C. difficile* infection has been relapse, which is seen in about 20% of patients treated with metronidazole or vancomycin

Relapse is caused by the initial strain of *C. difficile*, but nearly half of patients experiencing relapse may be infected with new strains of *C. difficile*.

Fidaxomicin

- Feb. 2, 2011 -- A new antibiotic, fidaxomicin, is as effective as vancomycin in curing *C. diff* (*Clostridium difficile*) infections and may be better at reducing recurrences, new research shows.
- ''A cure -- that means getting over the diarrhea -- was the same for both, about 90%,'' says researcher Sherwood Gorbach, MD, chief medical officer at Optimer Pharmaceuticals, which funded the study. "However, for recurrences there was a 45% reduction with fidaxomicin [over vancomycin]."
- *C. diff* infections, which often occur after a person has taken antibiotics, affect the gastrointestinal tract and can be deadly. Recurrences are common and troublesome with *C. diff* infection, seen in 20% or 30% of patients.
- In the study, 13.3% of those on fidaxomicin had a recurrence during the four-week follow up, compared to 24% of those on vancomycin.
- Infections with *C. diff* have been on the rise in recent years, with the incidence more than doubling since 1996. According to some estimates, up to 3 million cases of the infection are diagnosed in the U.S. annually.

New Hypervirulent C diff strain in Australia RT244

- News in Science
- **Antibiotic overuse fuels gastro bug fear**
- Monday, 25 March 2013 Dani Cooper
  ABC
- Unnecessary: Overuse of antibiotics is leaving the door open for a deadly gastro bug to wreak havoc on our health system *(Source: iStockphoto)*
- **Related Stories**
- Three 'types' of human gut bacteria found, Science Online, 26 Apr 2011
- Human poo transplant treats gut-wrenching disease, Science Online, 19 Apr 2011
- Study confirms antibiotics mess with gut, Science Online, 14 Sep 2010
- Health warning Unnecessary use of antibiotics could unleash a killer hypervirulent strain of gastro bug in Australia, warn infectious disease experts.
- They are calling for heightened awareness of the latest strain of *Clostridium difficile* within the community, and broader testing by GPs to track its spread.
- Speaking as part of the annual scientific meeting of the Australian Society for Infectious Diseases (ASID) Dr Tony Korman, of Monash Health, and Professor Tom Riley, from the University of Western Australia, say the RT244 strain, which has arrived in Australia, shares many genetic features with RT027.
- It was outbreaks of RT027 in the UK, US, Europe and Canada from the mid-2000s that caused major panic in the health systems and front-page news.

Fecal Microbiota Transplantation

- Antibiotics and other factors disrupt the normal balance of colonic flora
- This reduces “colonization resistance” allowing pathogenic C difficile strains to grow and produce toxins
- Severe diarrhea and Pseudomembranous colitis then occurs
- Introducing normal flora by donor feces corrects the imbalance
- Normal bowel function is reestablished

Indications for FMT

- Recurrent or relapsing CDI (at least 3 mild to mod episodes and failure of a 6-8 wk taper with vancomycin or 2 episodes of severe CDI resulting in hospitalization)
- Moderate CDI not responding to standard therapy
- Severe CDI with no response to standard rx for 48 hr

Donor Selection

- Intimate contacts (eg spouse) share infectious risk factors which reduces risk of transmitting infectious agent.
- However unrelated donors equally effective

Donor Exclusion

- Absolute
  - HIV, hepatitis B or C or known exposure 4
  - High risk sexual behaviors
  - Use of illicit drugs
  - tattoo or body piercing within 6 mos
  - incarceration or history of this
  - current communicable disease
  - risk for Creutzfeldt-Jakob disease
  - travel within 6 mos to areas where diarrhea illness endemic

Donor Exclusion (cont)

- G I diseases such as IBD, IBS, colon cancer or chronic constipation or diarrhea
- Antibiotics within past 3 mos
- Major immunosuppressive agents
- Chemotherapy
- Recent ingestion of a potential allergen

Donor Testing

- Stool testing for c difficile toxin B by PCR
- Routine stool culture
- Fecal giardia and cryptosporidium antigen
- Acid fast stain for Cyclospora and isospora
- Ova and parasites
- Serology for HIV, hep A, B and C and syphilis
- Cost approx $400

Recipient Preparation

- Large volume bowel prep
- Loperamide is optional

Stool Preparation

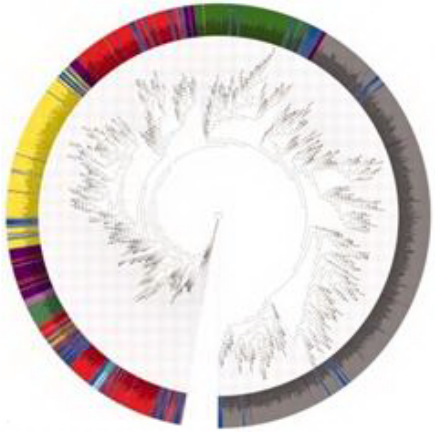
- Use as soon as possible(best fresh!)
- Use a hood if possible
- Use presevative free normal saline to dilute
- Use a standard blender and homogenize to slurry
- Filter with gauze and then use immediately
- Use 250-500mL and give by enema or cscope

What is link of antibiotics and CDI

- Antibiotics suppress and disrupt our microbiome that normally keep expansion of c difficile populations in check
- This allows c difficile bacteria to proliferate and produce toxins to cause colitis

Human Fecal Microbiota and CDI

- Complex ecosystem in symbiosis with host that is altered with antibiotics
  - Epithelium : immune cells : microbiota
    - 100 trillion bacteria colonize the gut
      - 200 to 1000 distinct bacterial species
    - All 3 play a defensive role
      - Essential for immune cell development and function
        - » Peyer's patches, lymph nodes, spleen, immunoglobulins
    - Gut microbes cross talk across intestinal epithelium and the immune system
    - Colonization resistance (competition for space and nutrients)
    - Products of bacterial metabolism bactericidal
      - Short chain fatty acids, reactive oxygen species
      - Bacteriocidin molecules



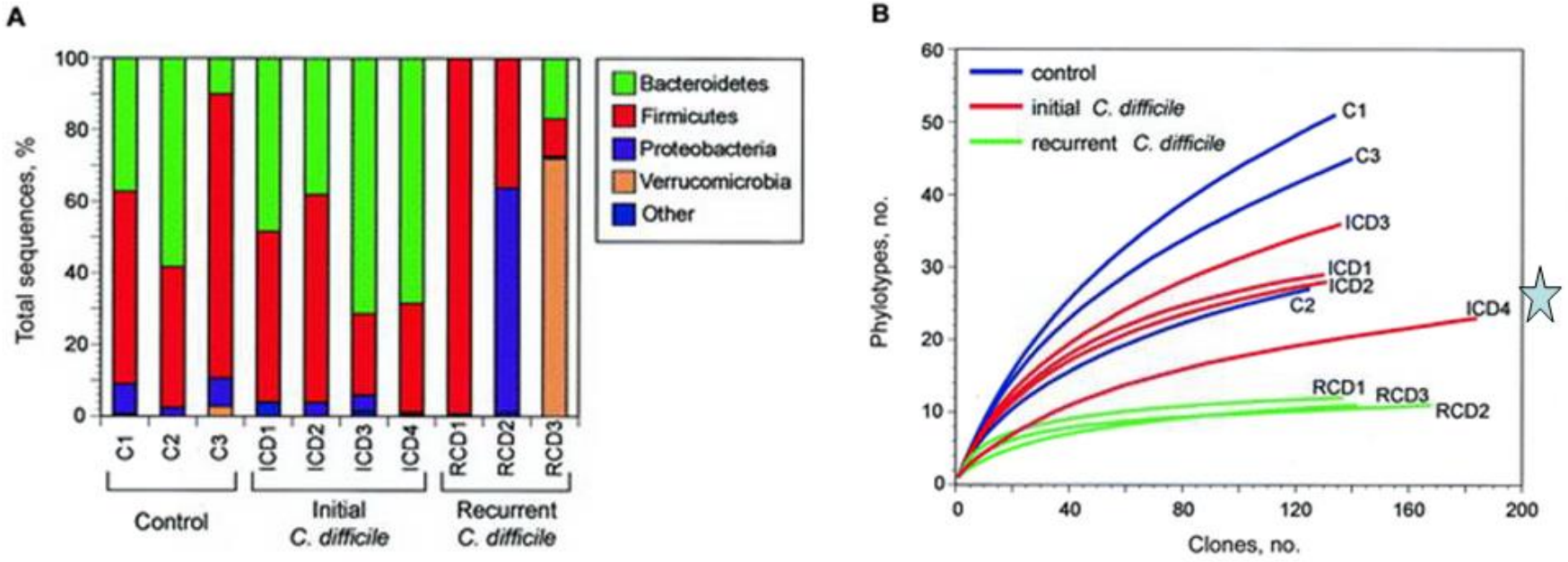
J Infect Dis 2008; 197:435- 8
Microbiol 2010; 156: 3216-3223
Curr Infect Dis Rep 2011;13:28–34

Intestinal Microbiota & Risk of CDI

- Antibiotic perturbation manifests as decreased colonization resistance of commensals
  - Short term: altered quantity and composition
  - May have a long term influence on return of gut flora to pre-treatment levels
    - Altered microbial diversity (Bacteroidetes / Firmicutes)
  - Antibiotic associated diarrhea
    - *C. difficile, K. oxytoca, C. perfringens, Salmonella spp.*, *Candida spp.*, and *S. aureus*

Curr Infect Dis Rep 2011;13:28–34

Decreased Diversity of Fecal Microbiome in Recurrent CDI



☆ Went on to relapse

J Infect Dis 2008;197:435

Fecal Transplant

Intestinal Microbiota Transplant (IMT)

Suspension of healthy donor intestinal microorganisms to restore the microbiota

- Used in recurrent CDI (also IBD / IBS)
- >2000 case series/reports (only 1 RCT)
  - Very effective
    - Disease resolution 92% cases
  - Variables
    - Route (enema vs colonoscope vs Jejunal)
    - Relationship of donor
    - Volume of infusion
    - Treatment prior to IMT

Fecal Transplant
Intestinal Microbiota Transplant (IMT)

- Systematic review 1957-2011
  - Case series (67%) and case reports
    - Evaluable cases: 317 patients
      - All had recurrent/relapsing CDI (91%) or PMC (9%)
    - Route: enema (35%); gastroscope/NJ (23%)
    - Donor: relative (66%)
    - Infusions: 1-48 (50% received 1)
    - Diluent: NS (62%)

Fecal Transplant

Intestinal Microbiota Transplant (IMT)

92% resolution (89% after 1 treatment)

- Related (93%) vs unrelated (84%)
  - Spouse or partner 96%
- Water (98.5%) vs NS (86%)
  - Relapses water 8% vs NS 3%
    - Milk (94%), saline/metamucil (94%)
- 500cc volume (97%) vs <200cc (80%)
  - Higher relapse if <50g stool used
- Enema (95.4%) vs Cscope (88.7%) vs NG/NJ (76.4%)
- ADR: death (1%) due to CDI, IBS, constipation
  - Perforation (? Incidence)

Clin Infec Dis 2011; 53, 10: 994-1002

Provocative Probiotic Primary Prevention Studies for

Antibiotic-assoc Diarrhea (ADD) and *C. difficile* infection (CDI)

- Probiotic Drink* Study *Hickson M, et al. British Med J 2007;335:80*

	Placebo	Probiotic
AAD	34%	12%
CDI	17%	0%

- Probiotic Capsule† Dose-ranging Study *Gao XW, et al. Am J Gastro 2010;1055:1*

	Placebo	Probiotic-1	Probiotic-2
AAD	44%	28%	16%
CDI	24%	9%	1%

- Multiple criticisms of these studies; exclusion criteria, study blinding, & unexplained high rate of CDI in controls

Use of probiotic preparations to prevent C.difficile Associated Diarrhea

- RDBPCT N=135
- Age 64 all taking antibiotics
- 100 gm BID L. casei as drink
- Results:
  - AAD: 7/57 (12%) vs 19/56 (34%)
  - 21% relative risk reduction, NNT 5
  - C.diff 0/57 vs 9/53 (17%)

Hickson M, et al . BMJ 2007

- Meta-analysis 28 studies
- N=3818 patients
- Moderate quality of evidence
  - Probiotics decrease incidence of CDAD by 66%
  - No adverse influence by receiving probiotics

Ann Internal Medicine 2012

Where is the Microbiome

- Largest reservoir of bacteria reside in the gut
- 100 trillion microbial cells and 1,000 microbial species
- 3-4 pounds of microbes in our gut
- Human Micorbiome Project (HMP) is a special NIH study $170 million over 5 yrs to catalyze microbiome research! Still more research in Europe, Japan, Canada and others!

photo[1] - Windows Photo Gallery

Copy To Gallery | File | Fix | Info | Print | E-mail | Burn | Open

Diversity of our Microbiome

- Any 2 people can have 80-90% difference in kinds of microbes they carry!
- 4,000 different strains of bacteria found in navel of 200 people but not a single strain was common to everyone!

Our initial microbiome

- We leave the sterile uterus and enter the world collecting our microbiome and activate our immune system
- Babies born by C section are colonized by skin bacteria rather than vaginal bacteria.
- thought to explain why C section babies have slightly higher rate of asthma and allergies
- May an explanation for why one study found babies born by C section were twice as likely to be obese at age 3 than those born vaginally

Gut Microbiota and Diabetes

- Danish study found men with type 2 diabetes had different microbiota than similar men without diabetes
- Fecal transplants given to men with metabolic syndrome from lean men using enemas
- After 6 wks insulin resistance and triglycerides declined in those who got the lean men's bacteria!

Gut Microbiota and Obesity

- Lean people have a different gut microbiota from obese people
- When gut bacteria were transplanted from obese mice to germ free lean mice the germ free mice gained twice as much weight than if transplanted from lean mice!
- Gut microbiota specialize in “dietary energy harvest”
- Babies with higher levels of Bifidobacteria during 1st yr of life were less likely to be overweight at age 7 than those with lower levels
- Pregnant women who gained excess weight during pregnancies had different gut microbiota than pregnant women who were normal weight

Did You Know

- There are 23,000 genes in the human genome
- There are 8,000,000 genes in the human microbiome
- microbiome= bacteria, viruses, fungi and other microorganisms
- The vast majority of microbes you come in contact with are not disease bearing

Probiotics

- So called good bacteria
- Try to improve your gut bacteria by repopulating your intestinal tract and crowd out “bad bacteria” or restore balance
- May stimulate the immune system or produce anti bacterial compounds that control other bacteria

What are Prebiotics?

- Non-digestible food carbohydrates that stimulate growth or activity of bacteria in the digestive system in ways that can be beneficial to health
- They act as food for the probiotics
- Examples: may be found in fruits, legumes and whole grains. These contain nondigestible dietary fiber such as inulin

Probiotics General Guidelines

- One billion CFUs (colony forming units) daily believed to be adequate dose
- 30-40 billion CFUs in 6 oz yogurt
- Better to buy as a dairy product in grocery store due to FDA requirements that certain number of bacteria present (rather than as capsules)
- In some fermented products more bacteria present at expiration date than at beginning

Safety of Probiotics

- Multiple studies show excellent safety profile
- Exception is Saccharomyces boulardii (a yeast and not a bacteria) not to be given in first 4 wk after bone marrow transplant (aka Florastor). Should also be used with caution with immunosuppressed pts

More about S boulardii

- It reduces by about half the frequency of relapses in those pts with recurrent CDI especially in those given high dose vancomycin

What's in the name? Bacterium

- Bacterium has 3 parts to name
- Genus (like lactobacillus)
- Species (like acidophilus)
- Strain (like LA-5)

Bifidobacterium lactis DN-173010

- Found in Activia yogurt
- It helps move food faster through gi tract and helps with constipation

Lactobacillus casei DN-114001

- Found in Danactive
- Seems to help immunity
- People taking it recovered faster from infection

Christ hospital probiotic study

- To help reduce C diff infection rate in hospital
- Use probiotic Bio K
- Fermented milk containing 50 billion live bacteria in 98 cc bottle or
- 2 capsules containing 60 billion bacteria
- Bacteria include lactobacillus acidophilus and lactobacillus casei
- Give only to selected inpts on 7 south

Christ study (cont)

- Based on Bio K study in Quebec in 2004
- All hospitalized pts who were on antibiotics given the probiotic daily
- 94%decrease incidence of severe c diff and 73%decrease total incidence of c diff
- Joseph Levato Pharm D in charge

Lactobacilla rhamnosus 66

- Culturelle
- Helps curb diarrhea caused by antibiotics

What good do they do?

- Digest fiber and other components of food we cannot digest
- Synthesize vitamins that we may not get enough via our food
- Suppress microorganisms the cause disease
- Stimulate the immune system

Probiotics in Clinical Medicine: Two Schools of Thought !

Definition: " Microorganisms normally present in the human body that when delivered in adequate amounts, confer a health benefit on the host"

Quakery

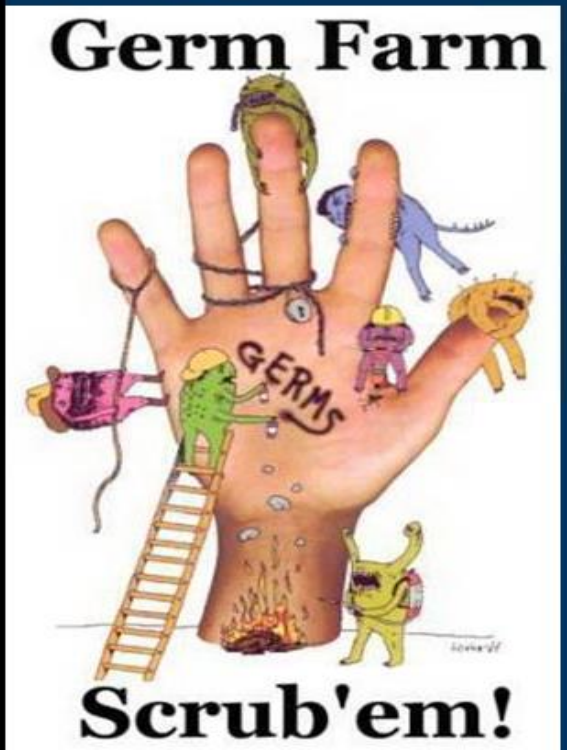
- Claims to cure "everything"
- Why so many strains ?
- How much is needed ?
- Review articles vs original articles
- Inconsistent studies
- Few ITT studies with tangible outcomes
- Marginal statistics
- Growing suspicion of "holoistic" medicine

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

Some say we should be killing our bacterial !!

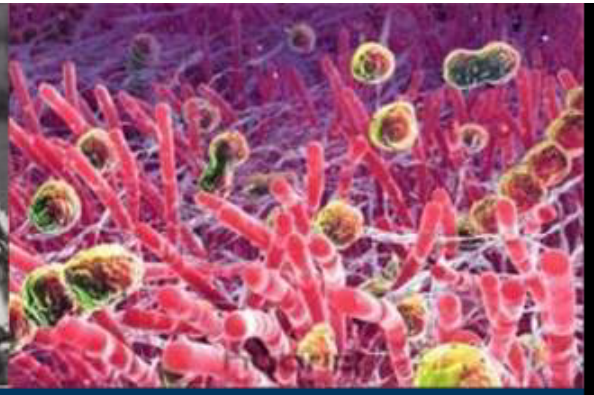


Gastroenterologist Survey of Probiotics

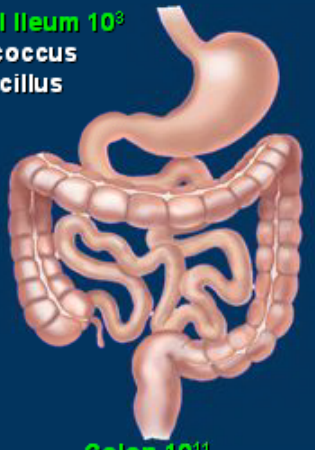
- Evaluate MD opinions regarding probiotics
- Large metropolitan area in midwest
- Results:
  - Safe for most patients 100%
  - 98% felt probiotics had a role in treating GI disease
  - 93% had patients currently taking probiotics
  - Most common bacteria used
    - Yogurt based, B.infantis (Align®),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

Where "man meets microbe" a dynamic interplay


Metchnikoff 1906

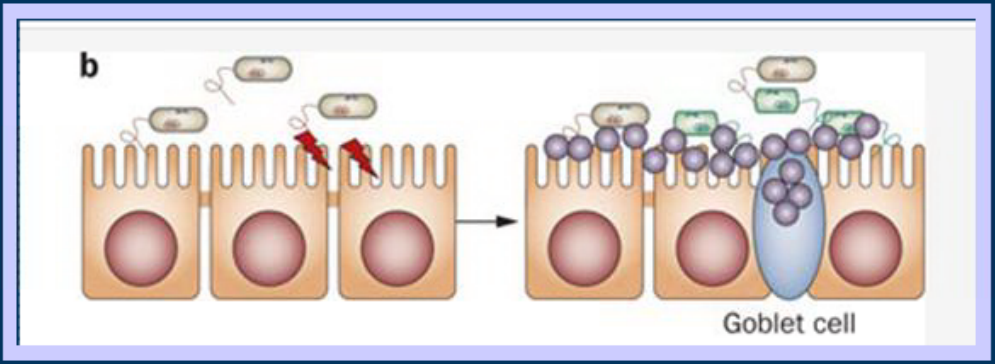


- Concepts are not new
  - Biblical references
  - Metchnikoff early 1900's
- 300 to 400 sq meter surface area
  - Surface area of a tennis court
- > 2 million genes in the bacterial genome vs 35,000 in the human
  - 100 trillion living bacteria in the human intestine
  - Over 500 species in human colon, non-culturalble
  - Each individual with own bacterial fingerprint
- 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: they partially explain bacterial community behavior and activation of virulence genes etc



Mechanisms:

Colonization Resistance
Antimicrobial Factors



Mechanisms

- Competitive inhibition
- Physical barrier (mucous)
- ↓ Adherence, attachment
- Produce bacteriocins
  - Defensins, Trefoil
  - Bind pathogens
- ↓ pH reduces growth
- Interfers 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

L. reuteri* inhibits *H. pylori

PM Sherman (NCP2009)
Morowitz M J (SCNA 2011)

L. reuteri* inhibits *Staph aureus

Potential applications for probiotics

- Metabolism
- Metabolism of dietary compounds
- in the gut lumen:
  - Lactose digestion
  - Lipid metabolism
  - Oxalate metabolism
- Composition and metabolic markers of the gut microbiota
  - Xenobiotics, phytochemicals
  - Indigestible dietary components
- Metabolic activity of gastrointestinal mucosa and liver
  - IBD and IBS
  - Inflammatory bowel diseases:
  - Crohn's disease
  - Ulcerative colitis
  - Pouchitis
  - IBS
- Allergic diseases
  - Eczema, atopic eczema
  - Allergic rhinitis
  - Asthma
- Reduction of risk factors of infection
  - Infectious diarrhea (acute and antibiotic-associated)
  - Traveler's diarrhea
  - Necrotozing enterocolitis (infants)
- Helicobacter pylori
- Respiratory tract infections (adults and children)
  - Ear, nose, and throat infections
- Infectious complications in surgical critically ill patients

Ritchie, M et al PLoS One 2012– Met Analysis of Probiotic Efficacy of GI Disease

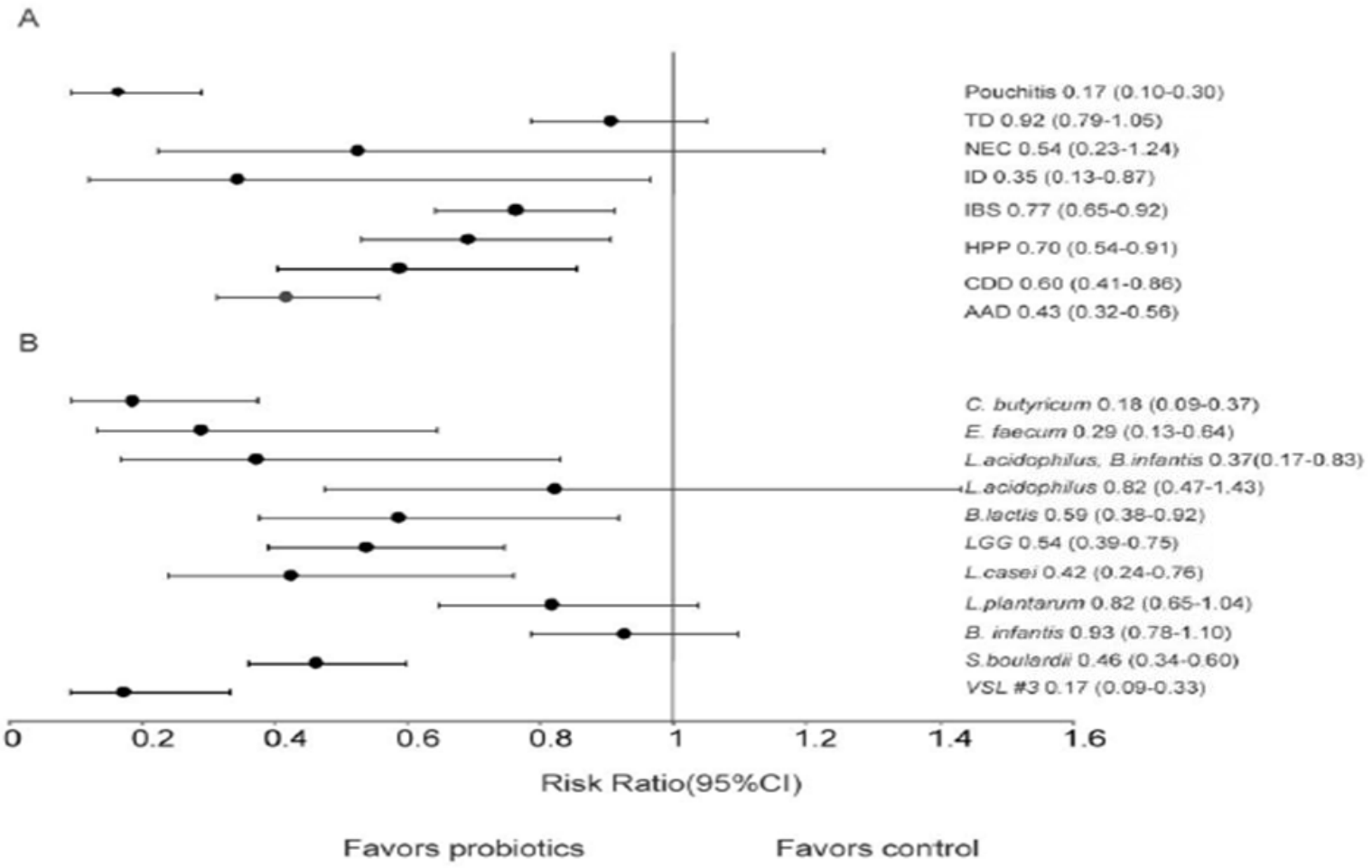
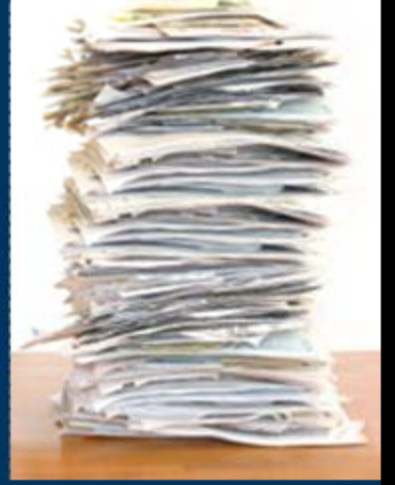


Figure 3. The effect size (risk ratio) for gastrointenstinal diseases and for probiotic species. (A) The effect size including the 95% confidence intervals for the total events of Antibiotic-associated diarrhea (AAD), *Clostridium difficile* disease (CDD), *Helicobacter pylori* positive (HPP)

Clinical Use:
Sorting evidence from myths !

Specific effects can be strain specific !



Level I evidence in:

- Infectious diarrhea (L GG)
- Prevention of traveller's diarrhea
- Prevention of pouchitis after total colectomy for UC
- Prevention of Ventilator Associated Pneumonia (VAP)
- Prevention of Necrotizing fasciitis in neonates
- Prevention of anti-biotic diarrhea

Level 2 evidence in:

- S.boulardii (with vanc) in preventing recurrent C.difficile
- Prevention of post op infections in liver transplant
- Prevention of post op infections in abdominal surgery

Probiotic based control of *H. pylori* infection

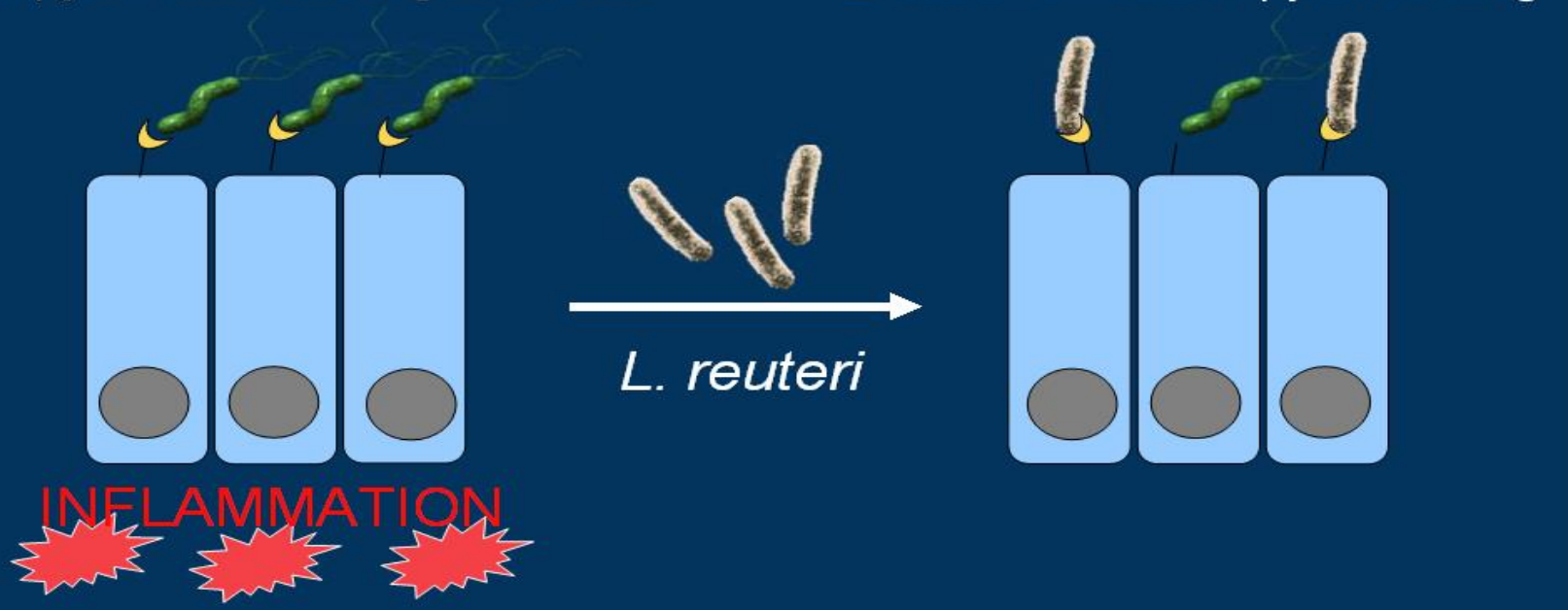
H. pylori infects at least half of the world's population. The prevalence among middle-aged adults is over 50% in many developing countries, as compared with 20% to 50% in industrialized countries. **WHO classifies H. pylori as class one carcinogen**

Suerbaum & Michetti NEJM 2002; 347:117

Specific probiotics have surface proteins that inhibit the binding of *H. pylori* in the stomach

H. pylori attached to gastric cells

L. reuteri inhibits *H. pylori* binding



•*Mukai et al. FEMS 32:105 (2002)*

Common Products

NCP 2012

Table 1. Common Probiotic Preparations Available in the United States

Trade Name	Active Ingredient	Form
Florastor	*Saccharomyces boulardii* 250 mg	Capsules
Florastor Kids	*S boulardii* 250 mg	Powder
Align	*Bifidobacterium infantis* 35264 (1×10^9 CFU)	Capsules
DanActive	*Lactobacillus casei* DN-114 001	Fermented milk
Activia	*Bifidobacterium lactis* DN-173 010	Yogurt
Fem-Dophilus	*Lactobacillus reuteri* RC-14, *Lactobacillus rhamnosus* GR-1	Capsules
Culturelle	*L rhamnosus GG* (1×10^{10} CFU)	Capsules
Culturelle for Kids	*L rhamnosus GG* (1×10^9 CFU)	Packets
Sustenex	*Bacillus coagulans* GBI-30, 6086 (BC30)	Capsules, chewies, and gummies
Floranex	*Lactobacillus acidophilus* (2×10^6 CFU)	Capsules
Lactinex	*L acidophilus* and *Lactobacillus helveticus* (*bulgaricus*)	Capsules and packets
Phillips Colon Health	*Lactobacillus gasseri*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*	Capsules

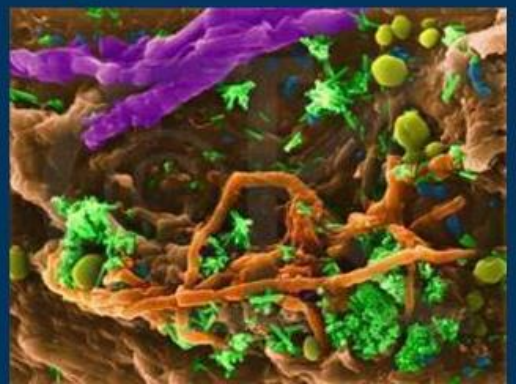
CFU, colony-forming units.

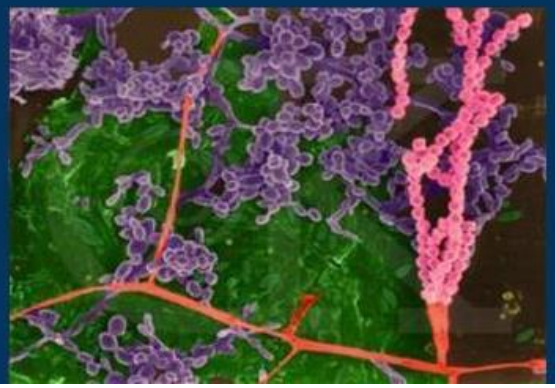
Is it time for a paradigm shift regarding bacteria ?

Are we making a leap of faith ?

Supply adequate viable beneficial bacteria or a substrate which enhances these specific beneficial bacteria instead of trying to eliminate the pathogen ?

"Bioecological control"






NEW!
probiotic
Lactose Free
THE GREEK GODS
Kefir
Low Fat Cultured Milk
HONEY
946 mL
WHOLE FOODS
NEW!
probiotic
Kefir

The Good, the Bad and the Ugly (Titles) 2:42

Ennio Morricone The Good, the Bad and the Ugly Soundtrack

Acknowledgement

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