Clostridium difficile infections
Crappy Options

Alexandru Petre David MD
Infectious Diseases

April 21, 2015
Objectives

- Microbiology
- Epidemiology
- Who is at risk?
- Pathogenesis
- Diagnosis
- Treatment
Historical Overview

- 1893: Finney first report of diphtheric colitis
- 1935: Hall and O’Toole: *B. difficile*
- 1950s and 1960s staphyloccal enterocolitis
- 1974: Tedesco: “clindamycin colitis”
- 1978: *C. difficile* – cause of clindamycin colitis
- 1981: FDA approves PO Vancomycin
- 1982: Use of metronidazole – never been FDA – approved
  - first tested – generic drug – no formal FDA approval process
  - 2 small randomized studies: Teasley 1983 and Wenisch 1996
- 2004: Hypervirulent NAP-1 strain
- 2010: IDSA guidlines
- 2011: Fidaxomicin FDA approval
Microbiology

- Obligately anaerobic, spore-producing, gram-positive rod
- 2-17 μm in length
- Fast-growing

- EM: flagellae or fimbria-like structures
- Stationary phase sigma factor: expressed at body temperature
- \textit{tcdC} gene – downregulates toxin production

- Toxin A
- Toxin B
- Binary toxin
Epidemiology

• Changing

• ? True increase in incidence

• ? Ascertainment bias – test more find more

• Better testing methods

• Increased quinolone use

• Hypervirulent strain
Epidemiology

• Definitions

• CDI case definition: ≥ 3 loose stools in 24h with a positive stool toxin assay or the presence of pseudomembranous colitis on endoscopy or histology

• Hospital - acquired: onset of symptoms occurred > 48 hrs after admission to, or less than 4 weeks after discharge from, a health-care facility

• Community – acquired: onset of symptoms occurred in the community or within 48 hrs of admission to a hospital, provided symptom onset was >12 weeks after the last discharge from the hospital

• Indeterminate: symptom onset occurred between 4 and 12 weeks from a hospital dismissal
Epidemiology

- CDC March 6, 2012 MMWR
- Population – based data from the Emerging Infections Program
- 56% aged > 65 years
- 94% of all CDIs were related to precedent and concurrent health-care exposure
- Of all health-care-associated CDIs: 75% onset outside hospitals
- 52% of the CDIs treated in hospitals are present on admission
- Potential source for intrahospital transmission
FIGURE 1. Percentage of *Clostridium difficile* infection (CDI) cases (N = 10,342), by inpatient or outpatient status at time of stool collection and type/location of exposures* — United States, Emerging Infections Program, 2010
Epidemiology

- Oct 2013 NEJM: David W. Eyre et al.

**Diverse Sources of *C. difficile* Infection Identified on Whole-Genome Sequencing**

- Describe the role of symptomatic patients in transmission

- 3.6-year study, 1200 patients in a defined geographic area with a typical incidence of the infection and standard infection-control practices

- ONLY 35% of cases were genetically related to at least one previous case (≤2 SNVs)

- 13% genetically related and involved ward contact

- 19% genetically related and involved “some sort of hospital contact”

- 3% genetically related: exposure to at least one intermediate host or source rather than direct contact
Epidemiology

- Importance:

- Substantial no of patients acquired CDI from sources other than symptomatic patients with positive C diff toxin on enzyme immunoassay

- Rapid benchtop sequencing – target cases to prevent further spread
Epidemiology

- July 2013 CID: Scott R. Curry et al.

**Use of Multilocus Variable Number of Tandem Repeats Analysis Genotyping to Determine the Role of Asymptomatic Carriers in Clostridium difficile Transmission**

- University of Pittsburgh Medical Center Presbyterian–Shadyside (UPMC)
- 3006 patient screened – 314 positive
- 56 incident cases of CDI classified as HA
- 17 (30%) associated with CDI patients
- 16 (29%) associated with carriers
  - 9 – non-ward transmissions
  - 2 – ward transmissions
  - 2 – environmental transmissions
  - 2 - indeterminate
Epidemiology

• Importance:

  • Supports the hypothesis that carriers contribute to transmission within hospitals
  • Screening and isolating patients with carriage to reduce CDI incidence
  • > 50% of CDI patients screened at > 14 days after their first positive toxin were negative by perirectal swab culture
  • Active surveillance screening program to determine the timing of contact precaution discontinuation

• Need for a controlled trial to evaluate the utility of screening for carriage with incident HA-CDI as the main study endpoint.
Who is at risk?

- **3 major factors:**
  - age
  - antibiotics
  - healthcare system

- **New categories:**
  - pediatric
  - pregnancy
  - out-patients

- **Other:**
  - obesity
  - critically ill burn patients
  - uremic patients
  - hematologic malignancies
  - gastrointestinal surgery
  - host IgG response
  - PPIs
Reservoirs

- 15% to 70% of stool of healthy neonates
- Variety of animals: cats, dogs, pigs, horses, elephants, etc.
- Soil, swimming pools, beaches, sea, river, tap water
- Healthy adults carriage
Who is at risk?


**Host and Pathogen Factors for Clostridium difficile Infection and Colonization**

- Age: for every additional year of age after age 18 the risk of HA-CDI increases by approx 2%

- NAP1 strain: independent risk factor for HA-CDI

- Colonization with a non-NAP1 strain: development of antibodies against toxin B that confer protection against acquisition of the NAP1 strain
Who is at risk?

- Virtually all antibiotic classes have been associated with CDI
- FLUOROQUINOLONES: clearly a risk factor for NAP1/O27 strain
- Don’t forget antineoplastic agents with modest antibacterial activity:
  - doxorubicin
  - cisplatin
  - cyclophosphamide
  - 5-fluorouracil
  - chlorambucil
  - methotrexate

KEY: alteration of the microecology of the gut
**Who is at risk?**

**TABLE 96-1** Antimicrobial and Chemotherapeutic Agents Associated with *Clostridium difficile* Diarrhea or Colitis

Data from Bartlett, Kelly et al., and Thielman and Guerrant.

<table>
<thead>
<tr>
<th>More Frequently Associated Agents</th>
<th>Less Frequently Associated Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins (especially second- and third-generation agents)</td>
<td>Ticarcillin-clavulanate</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>Ampicillin and amoxicillin</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Amphotericin B</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Rifampin</td>
</tr>
<tr>
<td>Other penicillins, including β-lactamase–stable penicillins</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td></td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Erythromycin and other macrolides</td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td></td>
<td>Sulfonamides</td>
</tr>
</tbody>
</table>
Arch Intern Med. 2010: Michael D. Howell et all.

Iatrogenic Gastric Acid Suppression and the Risk of Nosocomial *Clostridium difficile* Infection

Kaplan-Meier rates of nosocomial *Clostridium difficile* infection during the hospitalization
Iatrogenic Gastric Acid Suppression and the Risk of Nosocomial *Clostridium difficile* Infection

Rates of *Clostridium difficile* infection stratified by the type of antibiotics and acid-suppressive therapy. High-risk antibiotics included fluoroquinolones, cephalosporins, intravenous β-lactam/β-lactamase inhibitors, macrolides, clindamycin, and carbapenems. All other antibiotics were classified as low risk. H$_2$RA indicates H$_2$-receptor antagonist; and PPI, proton pump inhibitor.
Pathogenesis

- Key steps:
  - Disruption of normal colonic biota
  - Colonization with toxigenic *C. difficile*
  - Toxin A/B – cytoskeletal damage
  - Mucosal injury and inflammation

- Toxin A: 308-kDa enterotoxin – tcdA gene
- Toxin B: 269-kDa cytotoxin – tcdB gene
- Among most lethal bacterial toxins studied
- Binary toxin - ? Significance – NAP-1 strain
Pathogenesis

• The “hypervirulent” strain:

• The North American pulsed-field gel electrophoresis type 1 (NAP 1) or polymerase chain reaction (PCR) ribotype 027 and restriction endonuclease analysis (REA) group B1

• Originally reported in 2003 in Quebec

• Associated with higher CDI incidence and more severe and fatal disease
• 18 base-pair deletion in the negative regulatory element tcdC – translates into larger amount of toxin production
• Tendency to be fluoroquinolone resistant
• Binary toxin - ? Significance
Pathogenesis

PCR ribotype 078

- Keel et al. – the most common PCR ribotype among isolates from swine (83%) and calves (94%)
- Transmission from animals to humans via meat products

- 39-base pair deletion in the toxin regulatory gene tcdC
- Toxin production is less than that observed with ribotype 027 but significantly greater than in non-epidemic isolates
- Severe diarrhea in 40% cases
- Lower levels of complicated disease compared to 027
"He's smiling! — You must have given him the wrong food!"
Diagnosis
Diagnosis

**Clost. difficile colitis**

Superficial erosion of the mucosa of the colon, with an adherent "pseudomembrane" of fibrin, mucus, and inflammatory debris.
Diagnosis

- Clinical correlation
- No best testing scheme has been established
- Test only diarrheal stools – watery, foul odor

- EIA toxin test: 63-99% sensitivity, 75-100% specificity
- Culture for C.difficile: 89-100% sensitivity, 84-99% specificity
- Cell culture cytotoxin test: 67-100% sensitivity, 85-100% specificity

- PCR: sensitivity almost 100%; specificity 94-96%
  - detects the tcdB/tcdC gene encoding the toxin and not the toxin itself
  - ? Symptoms , ? genetic drift

- Endoscopy: 51% sensitivity, almost 100% specificity
The only rapid test which identifies three targets: Toxin B, Binary Toxin, and \textit{tcdC} deletion.

### XPERT \textit{C. difficile} RESULT ALGORITHM

<table>
<thead>
<tr>
<th>Xpert \textit{C. difficile} result</th>
<th>Individual Targets and Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxin B+</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>–</td>
</tr>
<tr>
<td>\textit{C. difficile} positive</td>
<td>+</td>
</tr>
<tr>
<td>\textit{C. difficile} positive</td>
<td>+</td>
</tr>
<tr>
<td>\textit{C. difficile} positive</td>
<td>+</td>
</tr>
<tr>
<td>Epidemic \textit{C. difficile} positive</td>
<td>+</td>
</tr>
</tbody>
</table>

### PATHOGENICITY LOCUS

- \textit{tcdD} (Regulator)
- \textit{tcdB} (Toxin B)
- \textit{tcdE}
- \textit{tcdA} (Repressor deletion 117)
- \textit{tcdC}

- \textit{cdtR} (Regulator)
- \textit{cdtA} (Binary Toxin)
- \textit{cdtB}
Diagnosis

• Clinical features:

- Diarrhea + history of antibiotic use
- Watery stools, mucoid stools, soft stools with foul odor
- Abdominal cramps (22%), fever (28%)
- Leukocytosis (50%)
- Hypoalbuminemia
- Acute kidney injury

- Severe/complicated: paralytic ileus with toxic megacolon
TABLE 3. Recommendations for the Treatment of *Clostridium difficile* Infection (CDI)

<table>
<thead>
<tr>
<th>Clinical definition</th>
<th>Supportive clinical data</th>
<th>Recommended treatment</th>
<th>Strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial episode, mild or moderate</td>
<td>Leukocytosis with a white blood cell count of 15,000 cells/µL or lower and a serum creatinine level less than 1.5 times the premorbid level</td>
<td>Metronidazole, 500 mg 3 times per day by mouth for 10–14 days</td>
<td>A-I</td>
</tr>
<tr>
<td>Initial episode, severe*</td>
<td>Leukocytosis with a white blood cell count of 15,000 cells/µL or higher or a serum creatinine level greater than or equal to 1.5 times the premorbid level</td>
<td>Vancomycin, 125 mg 4 times per day by mouth for 10–14 days</td>
<td>B-I</td>
</tr>
<tr>
<td>Initial episode, severe, complicated</td>
<td>Hypotension or shock, ileus, megacolon</td>
<td>Vancomycin, 500 mg 4 times per day by mouth or by nasogastric tube, plus metronidazole, 500 mg every 8 hours intravenously. If complete ileus, consider adding rectal instillation of vancomycin</td>
<td>C-III</td>
</tr>
<tr>
<td>First recurrence</td>
<td>...</td>
<td>Same as for initial episode</td>
<td>A-II</td>
</tr>
<tr>
<td>Second recurrence</td>
<td>...</td>
<td>Vancomycin in a tapered and/or pulsed regimen</td>
<td>B-III</td>
</tr>
</tbody>
</table>

* The criteria proposed for defining severe or complicated CDI are based on expert opinion. These may need to be reviewed in the future upon publication of prospectively validated severity scores for patients with CDI.
Size matters

Vancomycin

Metronidazole
CDI recurrence

• Frequency: 25-30 %
• Factors:
  - age > 65
  - need for antibiotics during CDI treatment
  - NAP-1
  - severe underlying medical conditions

• One episode of recurrent CDI – 45-65% of additional episodes

• Differential: IBS, small bowel overgrowth, lactase deficiency

• Why?
  - not fully understood
  - persistent spores
  - lower anti-toxin antibody levels
  - antibiotic resistance – not a factor
**Diagram and Text**

**Vitamin A**

1. Diet

2. Microbiota

3. Unmodified dietary components

5. Dietary components modified by microbiota (acetate)

4. Microbial signals (MAMPs)

6. SFB

**Metabolic signals**

**Classical innate signals**

- GPR43
- Other metabolite sensors?
- IL-1β
- Pro-IL-1β
- NF-κB
- MyD88
- TLRs

**Immunologically active nutrients and metabolites?**

**T cell**

- **mTOR**
  - Promotes T<sub>H1</sub>, T<sub>H2</sub>, T<sub>H17</sub> cell differentiation; inhibits T<sub>reg</sub> cell differentiation

- **RAR-RXR**
  - Promotes intestinal T-cell homing; promotes T<sub>H2</sub> and T<sub>reg</sub> cell differentiation

- **VDR-RXR**
  - Promotes T<sub>reg</sub> cell differentiation; inhibits T<sub>H1</sub> and T<sub>H17</sub> cell differentiation

- **AHR**
  - Promotes T<sub>H17</sub> and T<sub>reg</sub> cell differentiation

- **LXR and PPAR**
  - Control T-cell differentiation

**Antigen-presenting cell**

- **TLRs**
  - Inflammasomes

- **mTOR**
  - Modulates DC function and differentiation

- **RAR-RXR and VDR-RXR**

- **AHR**
  - Modulates DC differentiation

- **PKR**
  - Regulates inflammatory responses

- **GPR120**
  - Inhibits inflammatory responses in macrophages
Intestinal Microbiota and Host Physiology

Figure 2. Some examples of the effects of intestinal microbiota and host physiology. The intestinal microbiota can affect many aspects of normal host development and function. Members of the microbiota, with their various components or products of metabolism are shown in red. Microbial effects on the host are shown in green. Affected host phenotypes are shown in blue. AMP, antimicrobial peptides; DC, dendritic cells; Gm, Gram negative; HPA, hypothalamus-pituitary-adrenal; lap, intestinal alkaline phosphatase; PG, peptidoglycan; PSA, polysaccharide. From Sekirov et al (3).
### Table 1. Disorders associated with an altered intestinal microbiome

<table>
<thead>
<tr>
<th>Gastrointestinal</th>
<th>Non-gastrointestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholelithiasis</td>
<td>Arthritis</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Asthma</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td>Atopy</td>
</tr>
<tr>
<td>Idiopathic constipation(^{a})</td>
<td>Autism(^{a})</td>
</tr>
<tr>
<td>IBS(^{a})</td>
<td>Autoimmune disorders</td>
</tr>
<tr>
<td>IBD(^{a})</td>
<td>Chronic fatigue syndrome(^{a})</td>
</tr>
<tr>
<td>Familial Mediterranean Fever</td>
<td>Diabetes mellitus and insulin resistance(^{a})</td>
</tr>
<tr>
<td>Gastric carcinoma and lymphoma</td>
<td>Eczema</td>
</tr>
<tr>
<td>Recurrent <em>Clostridium difficile</em> infection(^{a})</td>
<td>Fatty liver</td>
</tr>
<tr>
<td></td>
<td>Fibromyalgia(^{a})</td>
</tr>
<tr>
<td></td>
<td>Hay fever</td>
</tr>
<tr>
<td></td>
<td>Hypercholesterolemia</td>
</tr>
<tr>
<td></td>
<td>Idiopathic thrombocytopenic purpura(^{a})</td>
</tr>
<tr>
<td></td>
<td>Ischemic heart disease</td>
</tr>
<tr>
<td></td>
<td>Metabolic syndrome(^{a})</td>
</tr>
<tr>
<td></td>
<td>Mood disorders</td>
</tr>
<tr>
<td></td>
<td>Multiple sclerosis(^{a})</td>
</tr>
<tr>
<td></td>
<td>Myoclonus dystonia(^{a})</td>
</tr>
<tr>
<td></td>
<td>Obesity</td>
</tr>
<tr>
<td></td>
<td>Oxalic acid kidney stones</td>
</tr>
<tr>
<td></td>
<td>Parkinson's disease(^{a})</td>
</tr>
</tbody>
</table>

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome. 
\(^{a}\)Indicates some reports on transient or long-term improvement or cure with fecal microbiota transplant.
CDI recurrence

Management

Antibiotic therapy:

- **Vancomycin** – tapered or pulse dosing
- Sequential therapy with vancomycin followed by *rifaximin*
- **Fidaxomycin**
  - phase III trial – Louie T. NEJM 2011 – clinical cure rates with fidaxomicin and vancomycin were similar but recurrence was less often with fidaxomicin among patients with non-NAP1 strains (10 % vs 28 %)

Nonantibiotic therapy:

- **Probiotics** – inconclusive regarding benefit
- **Monoclonal antibodies** – not yet available for routine clinical use
- **Diverting loop and colonic lavage** – reduced mortality and colon preservation
- Fecal bacteriotherapy
FMT

• Indications:

  Recurrent or relapsing CDI
  - 3 or more episodes of mild to moderate CDI and failure of a 6- to 8-week taper with vancomycin with or without alternative antibiotics agents
  - At least 2 episodes of CDI that result in hospitalization and are associated with significant morbidity

  Moderate CDI not responding to standard therapy for at least 1 week

  Severe CDI with no response to standard therapy after 48 hours

Federal regulations:
- FDA ruled in 2012 that human feces constitutes a drug
- Under jurisdiction of pharmacy
- As of 2013 treatment IND – not required
- Requires patient consent

Many concerns: adverse events, autoimmunity, etc.
Patient eligibility determination:

History and physician exam

Serologic testing (optional)
- HIV
- Hep A total
- Hep BsAg, Hep BsAb and core Ab
- Hep C Ab
- RPR quant

Female subjects and pregnancy
Donor Eligibility Determination:

Donor selection and screening
- Preferably a relative
- Over the age of 18

Donor interview and screening Questionnaire
- Presence of medical or infectious conditions
- Donor History Questionnaire (DHQ)
FMT

DHQ will be used to exclude donors with these and other risk factors:

1. High risk sexual behaviors
2. Known exposure to HIV or viral hepatitis within the previous 12 months.
3. Being held in a correctional facility for more than 72 hours in the last 12 months
4. Use of intravenous drugs or intranasal cocaine
5. Recent tattoo or body piercing
6. Use of antibiotics within the past 3 months
7. Recent transfusion, transplant or skin graft
8. Risk factors for variant Creutzfeldt-Jakob disease
9. Use of antibiotics a period of 3 months.

Needs to be completed within 30 days before FMT
Donor Laboratory Testing

- Donor screening: Blood (within 30 days of procedure)
  - Negative for Hepatitis A, B, and C
  - Negative for HIV-1 and HIV-2
  - Negative for syphilis

- Donor screening: Stool (within 30 days of procedure)
  - Routine stool culture for bacterial pathogens is negative
  - Light microscopy examination of stool for ova and parasites is negative
  - C. difficile toxin is negative (order must indicate this is for donor screening)
Collection and preparation of stool:

On the day of fecal donation ask donor

- Fever, vomiting, diarrhea within the last 30 days
- Ingestion of potential allergen

Stool collection:

- Donor asked to provide fresh stool in clean container on day of colonoscopy
- Stool collection should be within no more than 6 hours of planned procedure, preferably within two hours whenever possible
- Donor may use tap water enema if unable to spontaneously provide specimen
- Specimen is labeled with donor’s name, MRN, and date and time of collection
FMT

Location & preparation of processing area:

1. Stool will be processed in a designated area within the Laboratory.
2. Standard precautions will be used during processing (gown, gloves, eye protection).
3. Clean counter surface will be covered with a disposable pad.
4. After the FMT, all surfaces will be wiped with hospital-approved disinfectant solution.

Preparation materials:

1. Non-bacteriostatic normal saline
2. Sterile container with lid (optional – a blender) to homogenize donor stool with saline
3. Clean gauze pieces (optional - disposable sieve) for filtration
4. Clean plastic spoon
5. 60 cc disposable slip (catheter)-tip syringes
6. Enema bottles
Preparation method:

1. Available quantity of stool (typically 50 grams) will be added to non-bacteriostatic normal saline (250-500 ml) in sterile container.
2. The mixture can be homogenized by shaking or using a blender.
3. This solution will then be filtered if necessary to remove larger articulate matter.
4. The fecal suspension will be drawn into aliquots of 60 cc slip (catheter) tip syringes or enema bottles for infusion.
5. Syringes, appropriately labeled, will be delivered to procedure area for administration.
Patient preparation:

1. Patients will have completed at least a 10 day course of vancomycin (or other anti-CDI therapy such as fidaxomicin or metronidazole) for the most recently diagnosed acute CDI prior to undergoing FMT.
2. To prevent disease relapse while awaiting FMT, anti-CDI therapy will be continued by patients up until 2-3 days prior to scheduled procedure.
3. For endoscopic administration of FMT, the subject will be prepped with standard bowel purge administered the day before the procedure.
4. 1-2 hours before colonoscopy/NGT infusion the patient may take 2 loperamide tablets to aid in retention of administered donor stool.
5. For NGT infusion patient will have nasogastric/orogastric tube insertion prior to infusion.
FMT Procedure

Methods of FMT infusion:

Colonoscopy
- Allows full endoscopic examination of the colon and exclusion of co-morbid conditions (such as IBD, microscopic colitis, malignancy, diverticulosis) which may have an impact on patient’s treatment or response to therapy.

Nasogastric tube
- This method may be preferable in patients who have already had recent endoscopic evaluation, in patients who prefer not to undergo endoscopy or in patients with significant co-morbidities and may not tolerate endoscopy.
## FMT

### WHAT IS CURRENT KNOWLEDGE

- The clinical and economic burden of *Clostridium difficile* infection (CDI) is significant.
- The overall efficacy of current antibiotics for treatment of recurrent CDI is suboptimal.
- Case reports and case series have suggested that fecal microbiota transplantation (FMT) may be a possible treatment for recurrent CDI.

### WHAT IS NEW HERE

- Based on the uncontrolled observational data, FMT appears to be effective in treating recurrent CDI but there are no published randomized-controlled trials.
- Lower gastrointestinal FMT delivery via colonoscopy or enema may be preferred to upper gastrointestinal delivery although randomized controlled trials are required to confirm.
“RePOOPulating” the gut

- Petrof E. Microbiome.2013
- Stool substitute preparation
- Purified intestinal bacterial cultures
- 33 isolates recovered from a healthy donor stool sample
- 2 patients infected with the hyper virulent c.difficile strain ribotype 078
- Colonoscopic infusions
- Both cured
- rRNA sequences found in the stool substitute constituted over 25% of the sequences up to 6 months after treatment

Advantages:
- Exact composition of the bacteria administered is known
- Bacterial species composition can be reproduced
- Pure culture more stable than stool
- Absence of viruses and other pathogens
Thank you!