Synthetic BIOLOGICS

SYN-004 (ribaxamase) PREVENTED *Clostridium difficile* INFECTION IN PATIENTS BEING TREATED WITH BETA-LACTAM ANTIBITOICS

John F. Kokai-Kun

6th International *C. difficile* Symposium

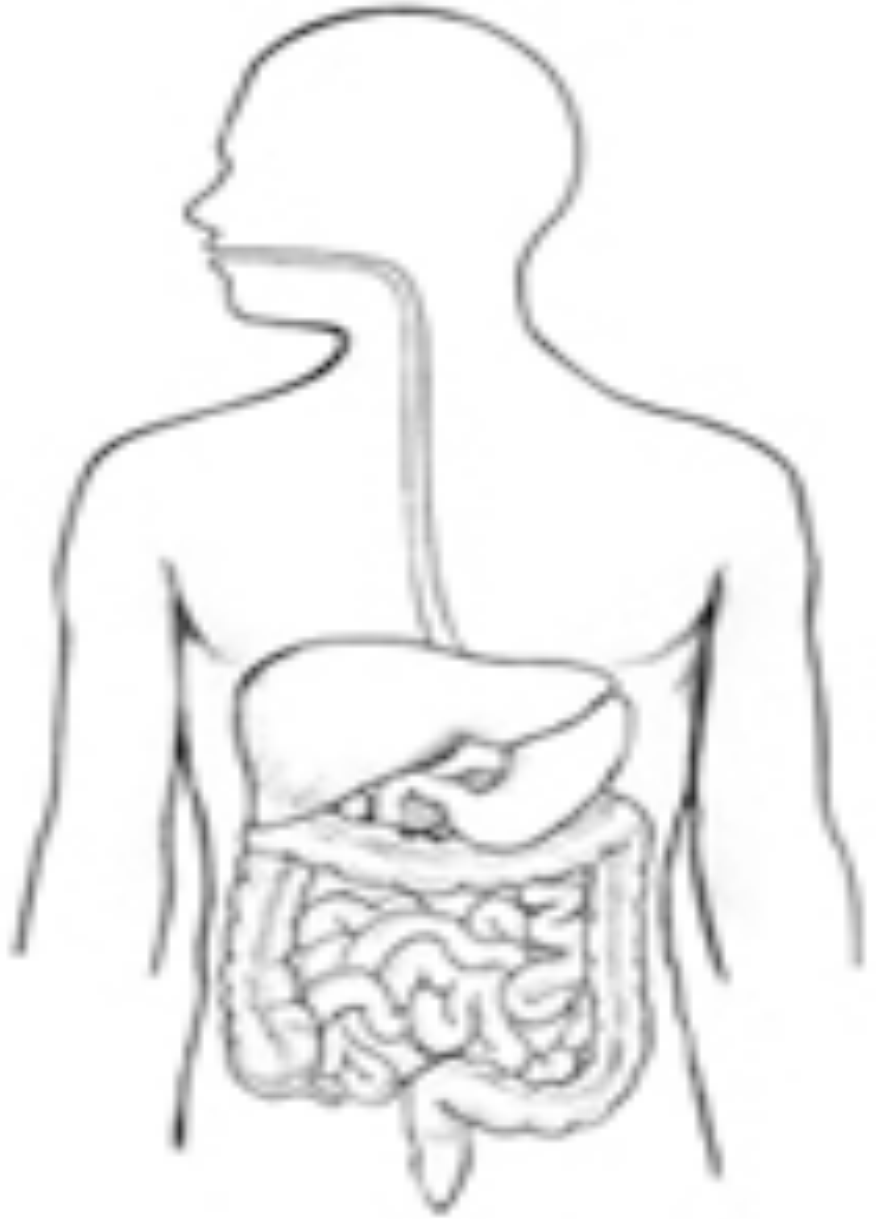
Bled, Slovania

September 12, 2018

Forward-Looking Statements

This presentation includes forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, on Synthetic Biologics' current expectations and projections about future events. In some cases forward-looking statements can be identified by terminology such as "may," "should," "potential," "continue," "expects," "anticipates," "intends," "plans," "believes," "estimates," "indicates," and similar expressions. These statements are based upon management's current beliefs, expectations and assumptions and are subject to a number of risks and uncertainties, many of which are difficult to predict and include statements regarding our timeline for our SYN-004 (ribaxamase) and SYN-010 clinical trials and reporting of data, the size of the market, benefits to be derived from use of SYN-004 (ribaxamase) and SYN-010, our anticipated patent portfolio, and our execution of our growth strategy. The forward-looking statements are subject to risks and uncertainties that could cause actual results to differ materially from those set forth or implied by any forward-looking statements. Important factors that could cause actual results to differ materially from those reflected in Synthetic Biologics' forward-looking statements include, among others, our product candidates demonstrating safety and effectiveness, as well as results that are consistent with prior results, our ability to initiate clinical trials and if initiated, our ability to complete them on time and achieve the desired results and benefits, our clinical trials continuing enrollment as expected, our ability to obtain regulatory approval for our commercialization of product candidates or to comply with ongoing regulatory requirements, regulatory limitations relating to our ability to promote or commercialize our product candidates for the specific indications, acceptance of our product candidates in the marketplace and the successful development, marketing or sale of our products, developments by competitors that render our products obsolete or non-competitive, our ability to maintain our license agreements, the continued maintenance and growth of our patent estate, our ability to become or remain profitable, our ability to establish and maintain collaborations, our ability to obtain or maintain the capital or grants necessary to fund our research and development activities, a loss of any of our key scientists or management personnel, and other factors described in Synthetic Biologics' annual report on Form 10-K for the year ended December 31, 2017, subsequent quarterly reports on Form 10-Qs and any other filings we make with the SEC. The information in this presentation is provided only as of the date presented, and Synthetic Biologics undertakes no obligation to update any forward-looking statements contained in this presentation on account of new information, future events, or otherwise, except as required by law.

Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection



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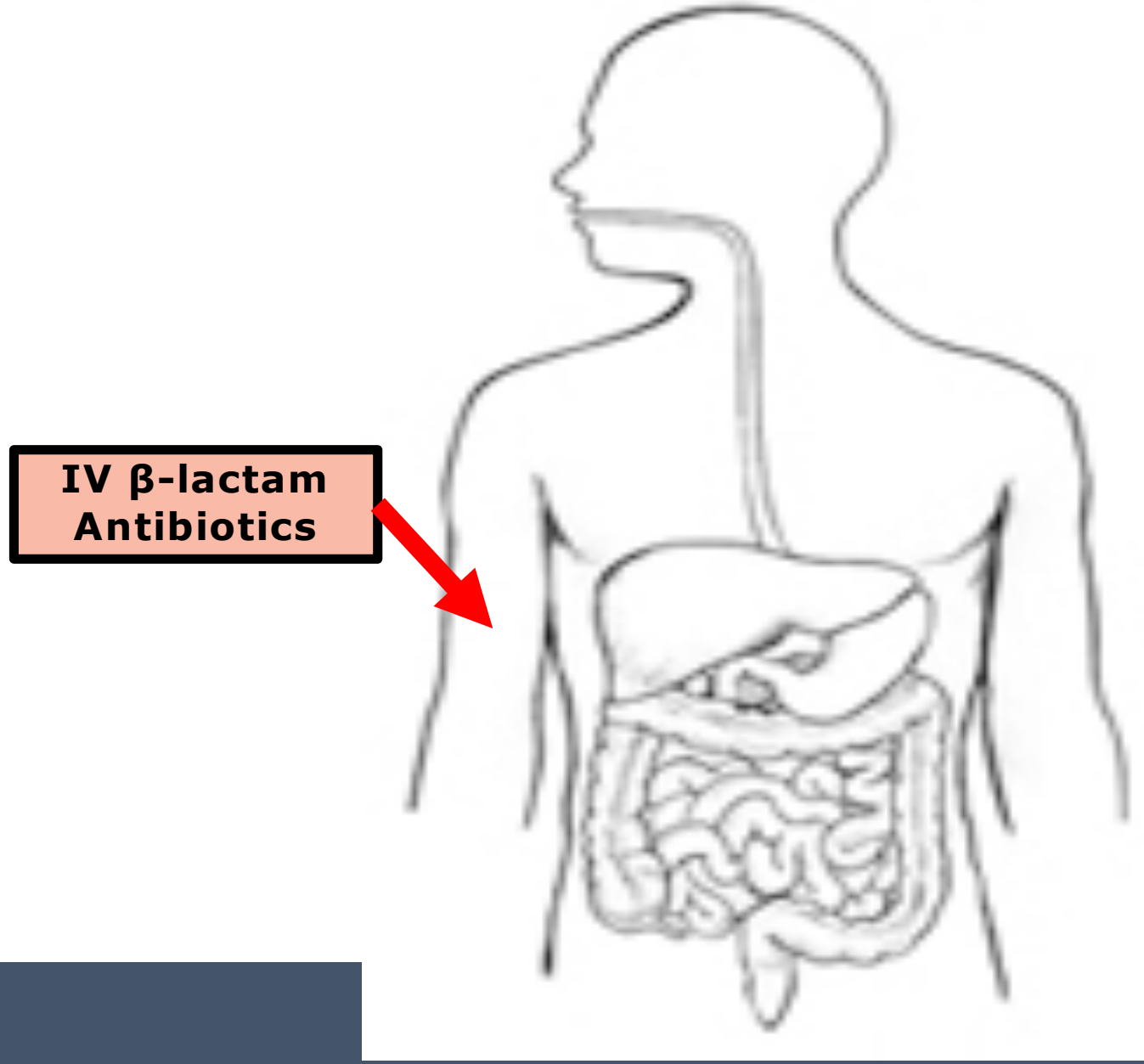

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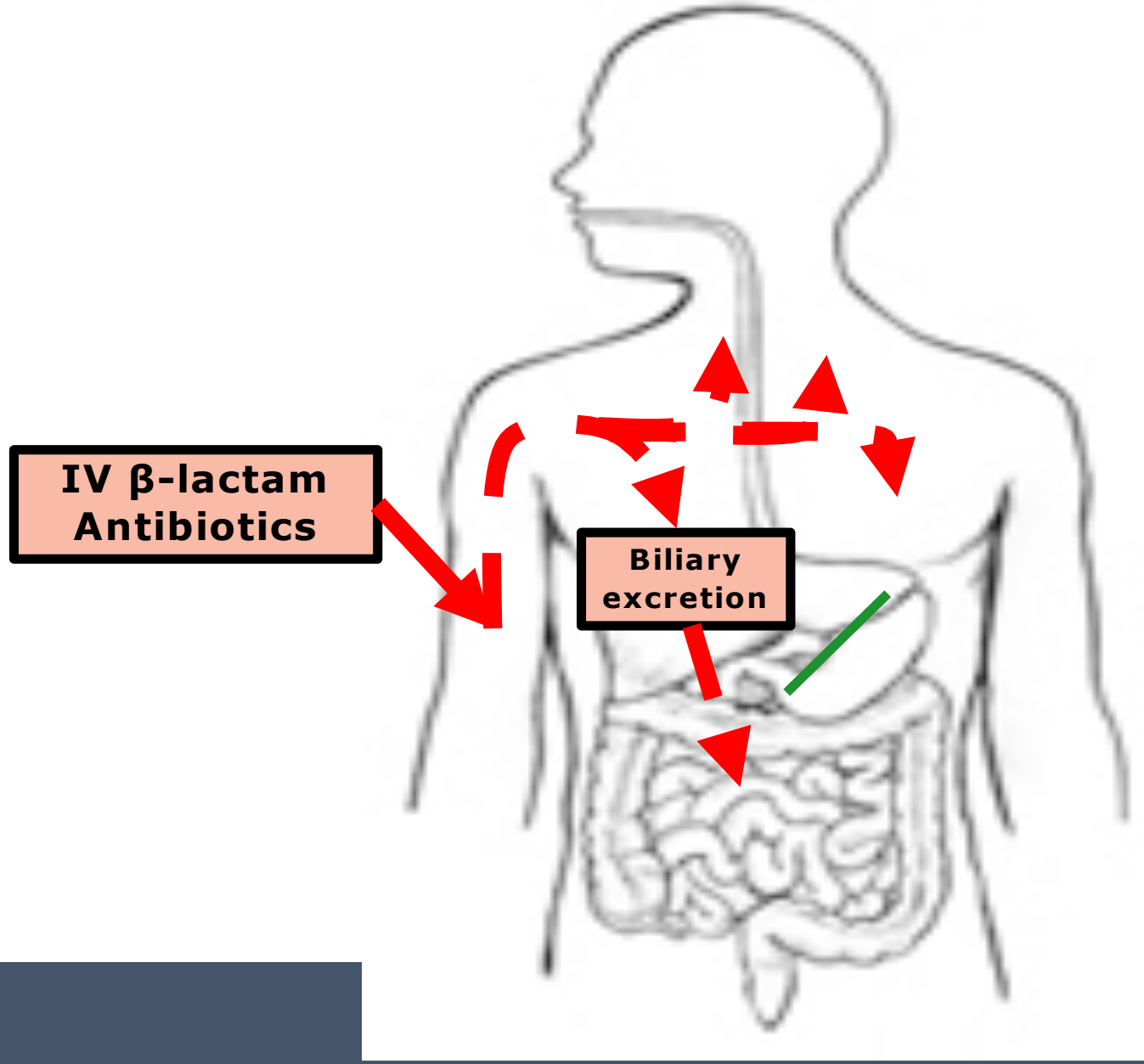

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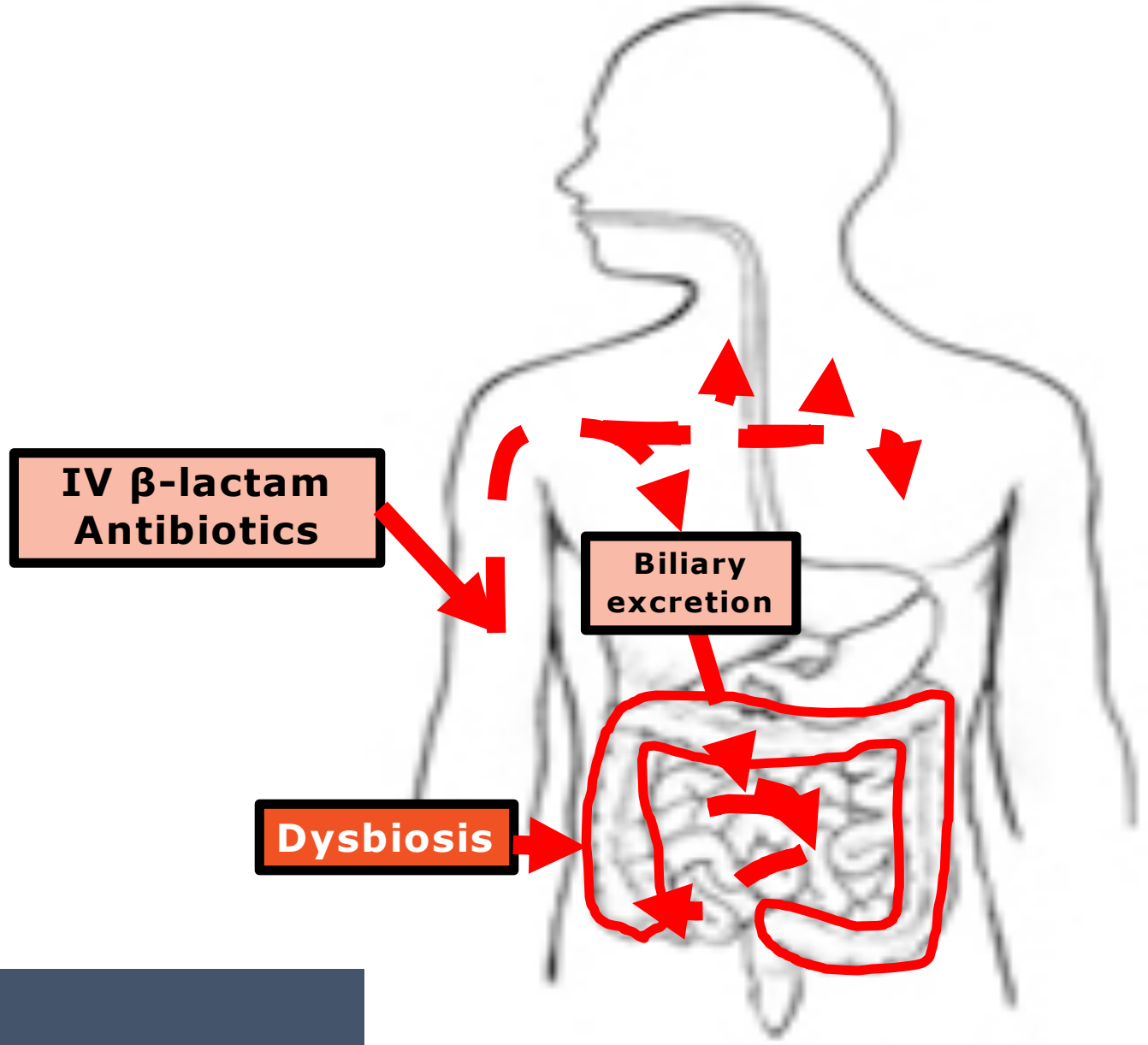

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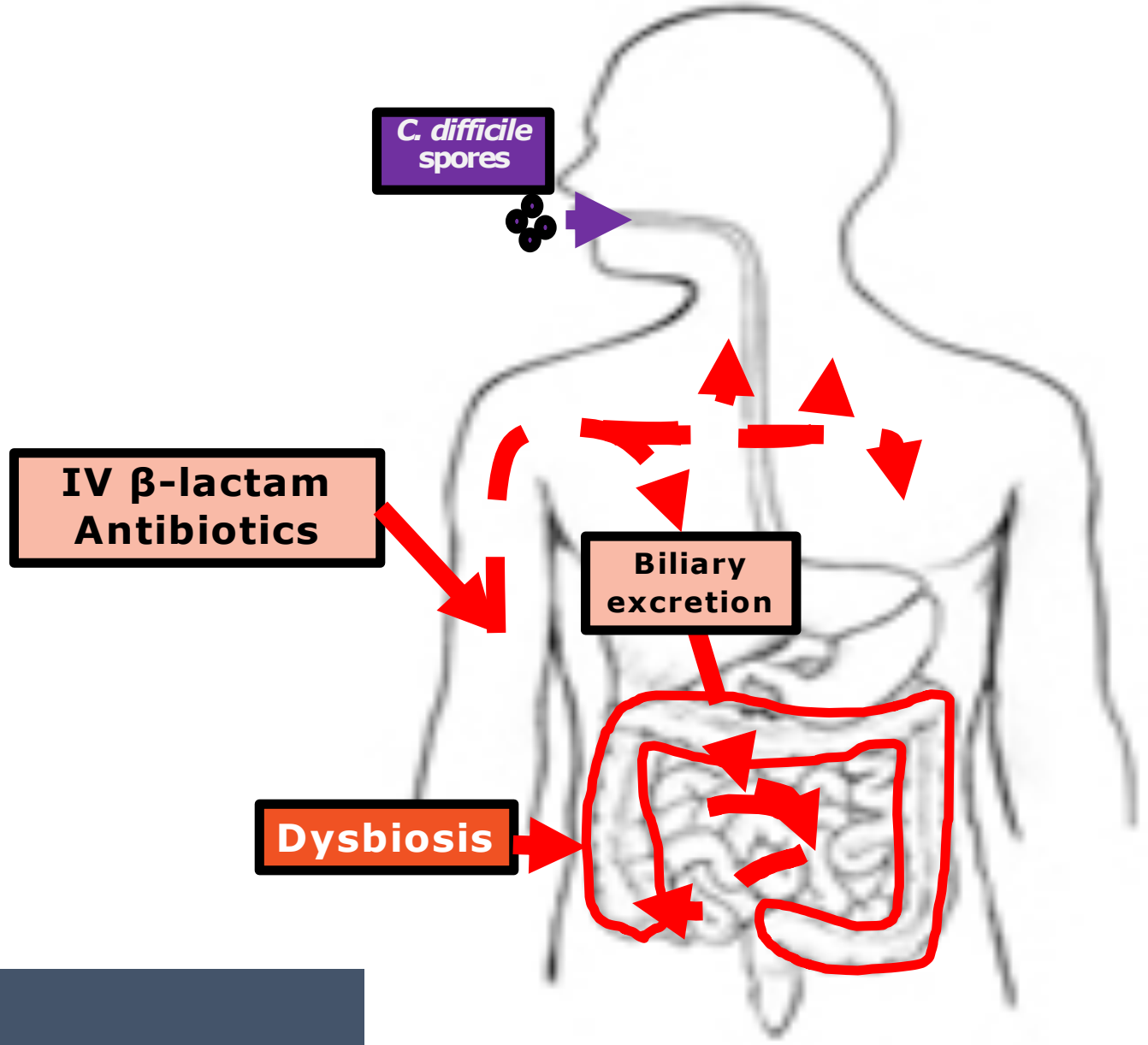

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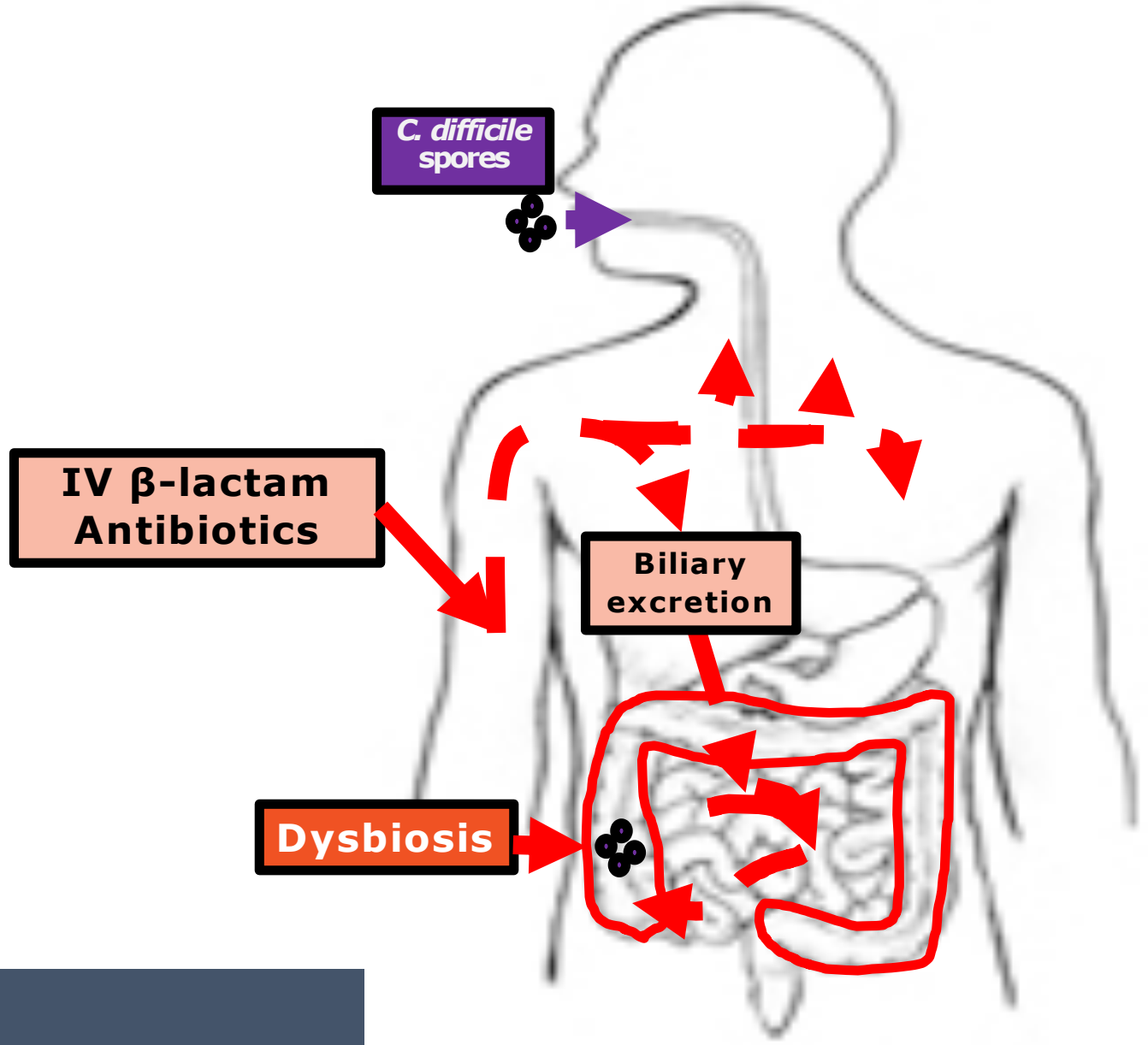

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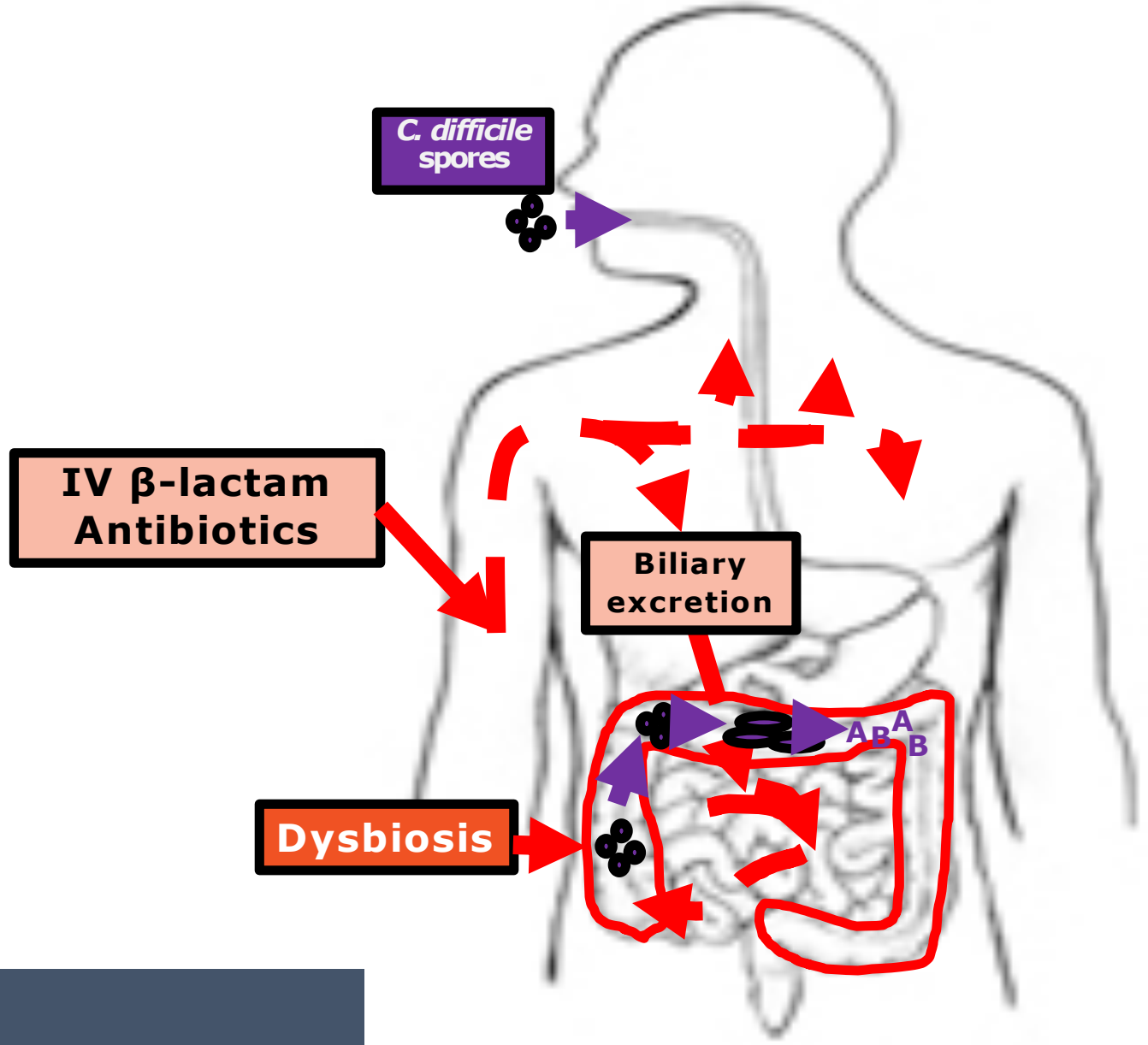

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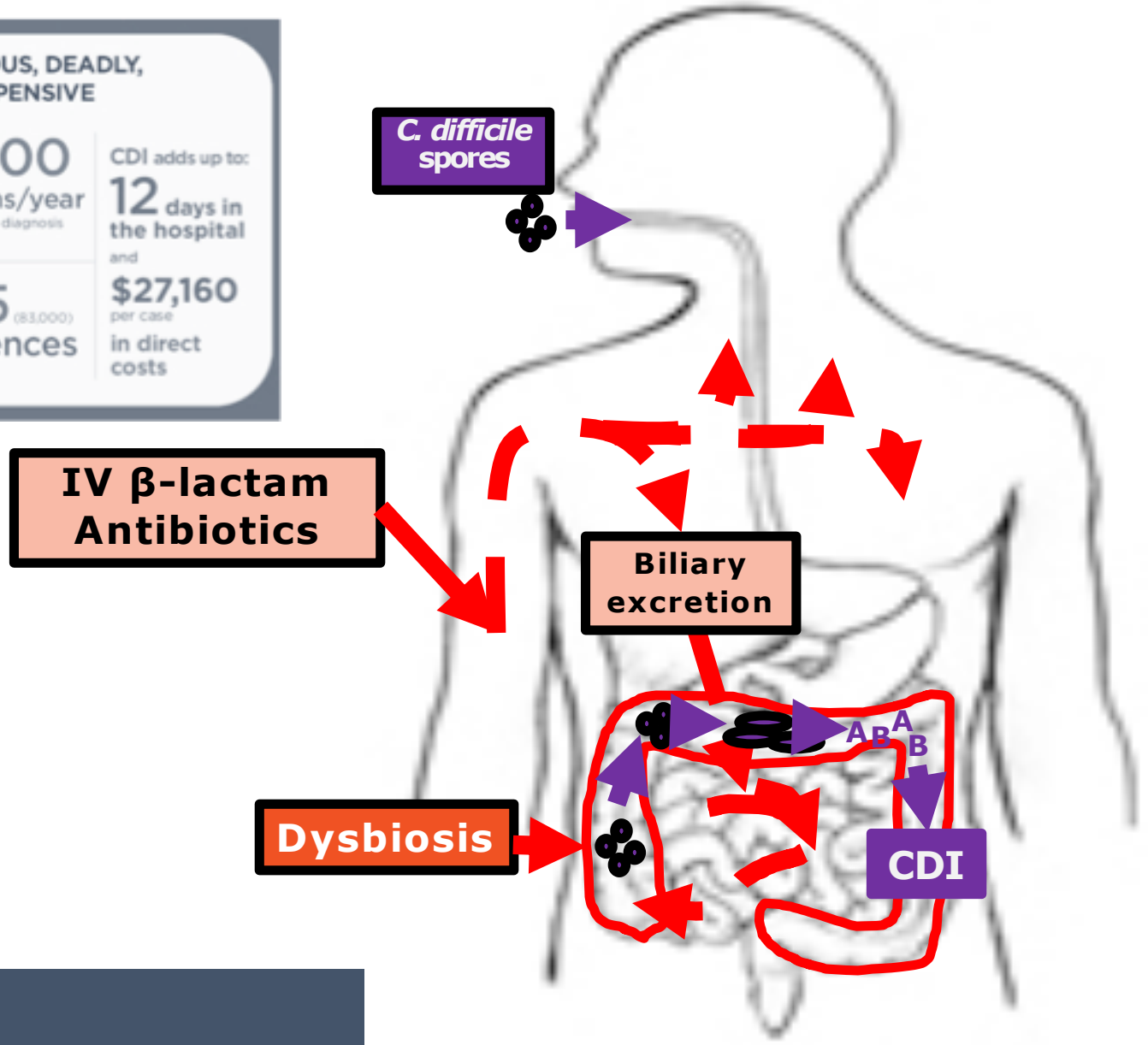
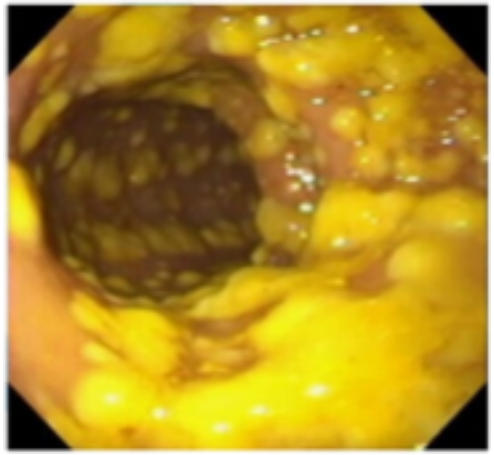
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection

CDI IS SERIOUS, DEADLY, AND EXPENSIVE

- **29,000** US deaths/year within 30 days of diagnosis
- **1 in 5** (83,000) recurrences within 2 months
- CDI adds up to: **12** days in the hospital and **$27,160** per case in direct costs





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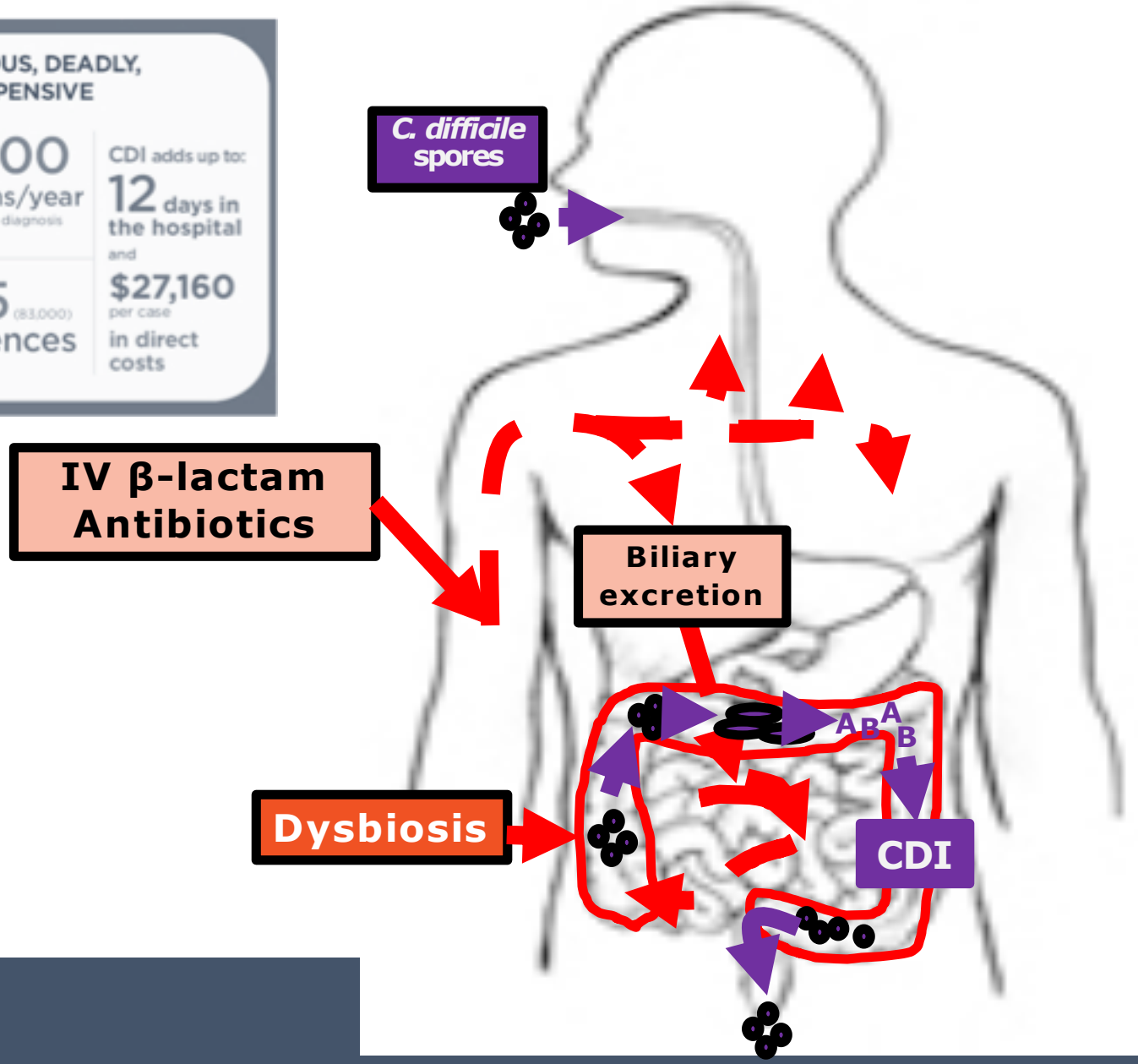

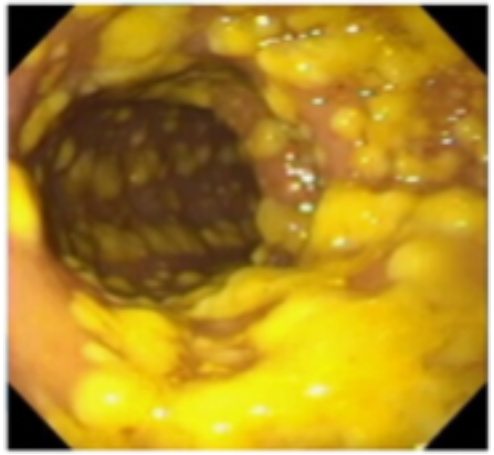

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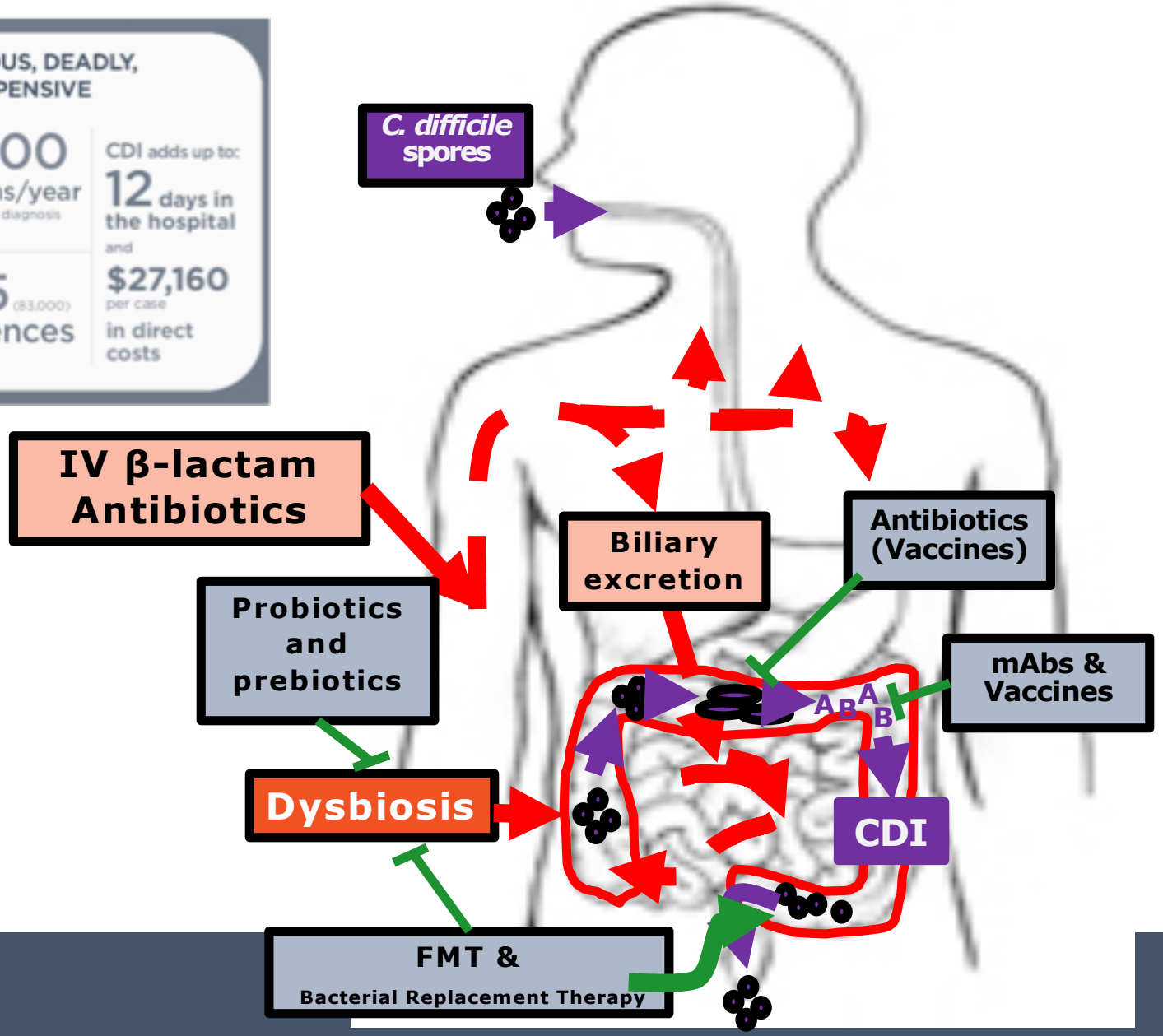

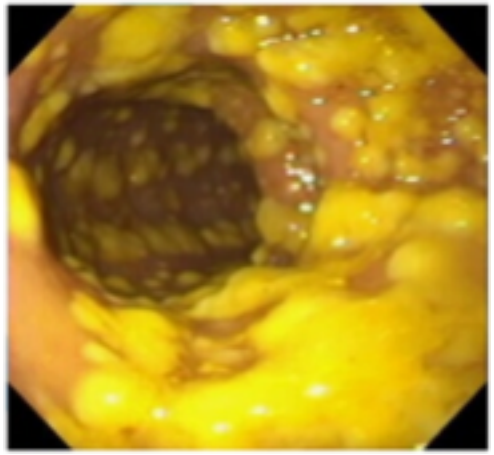

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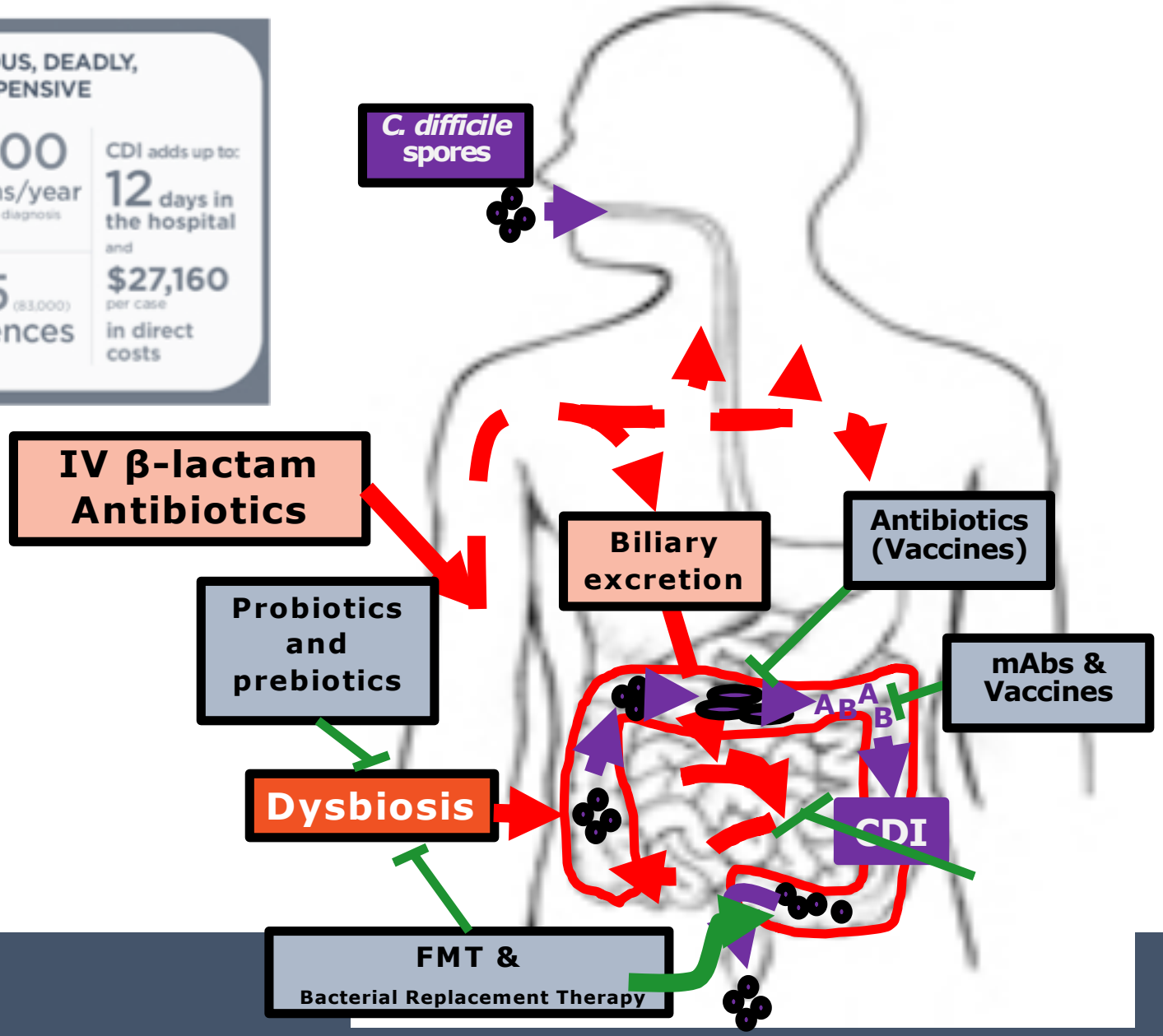

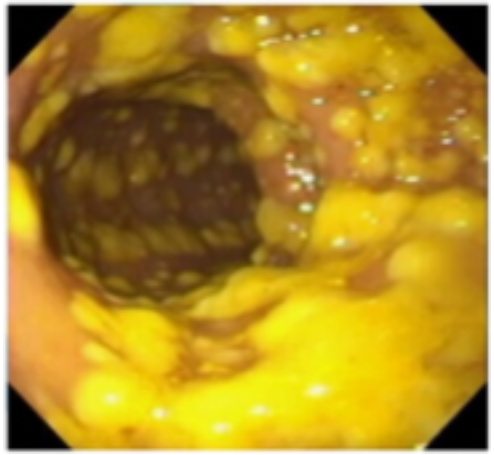

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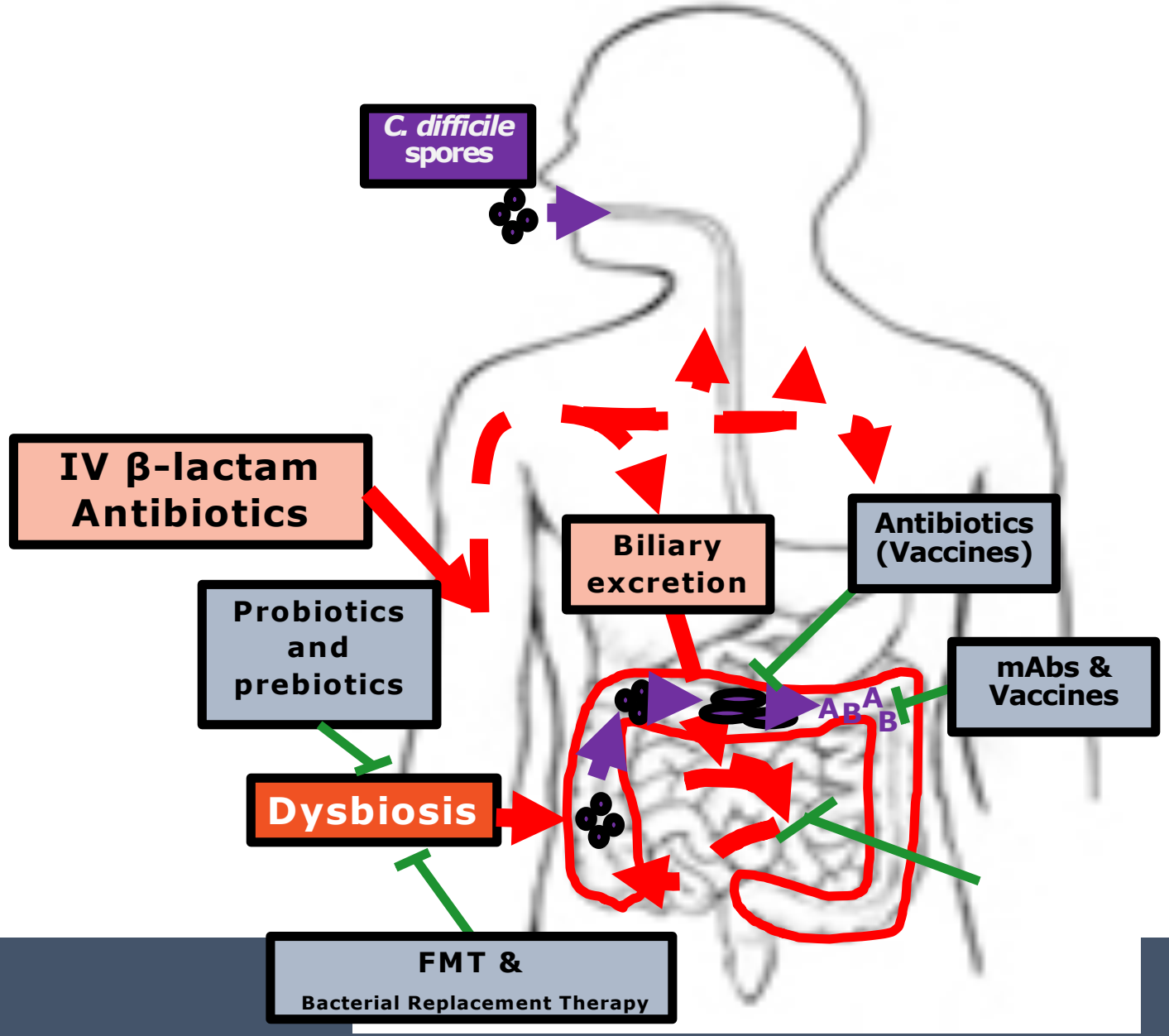

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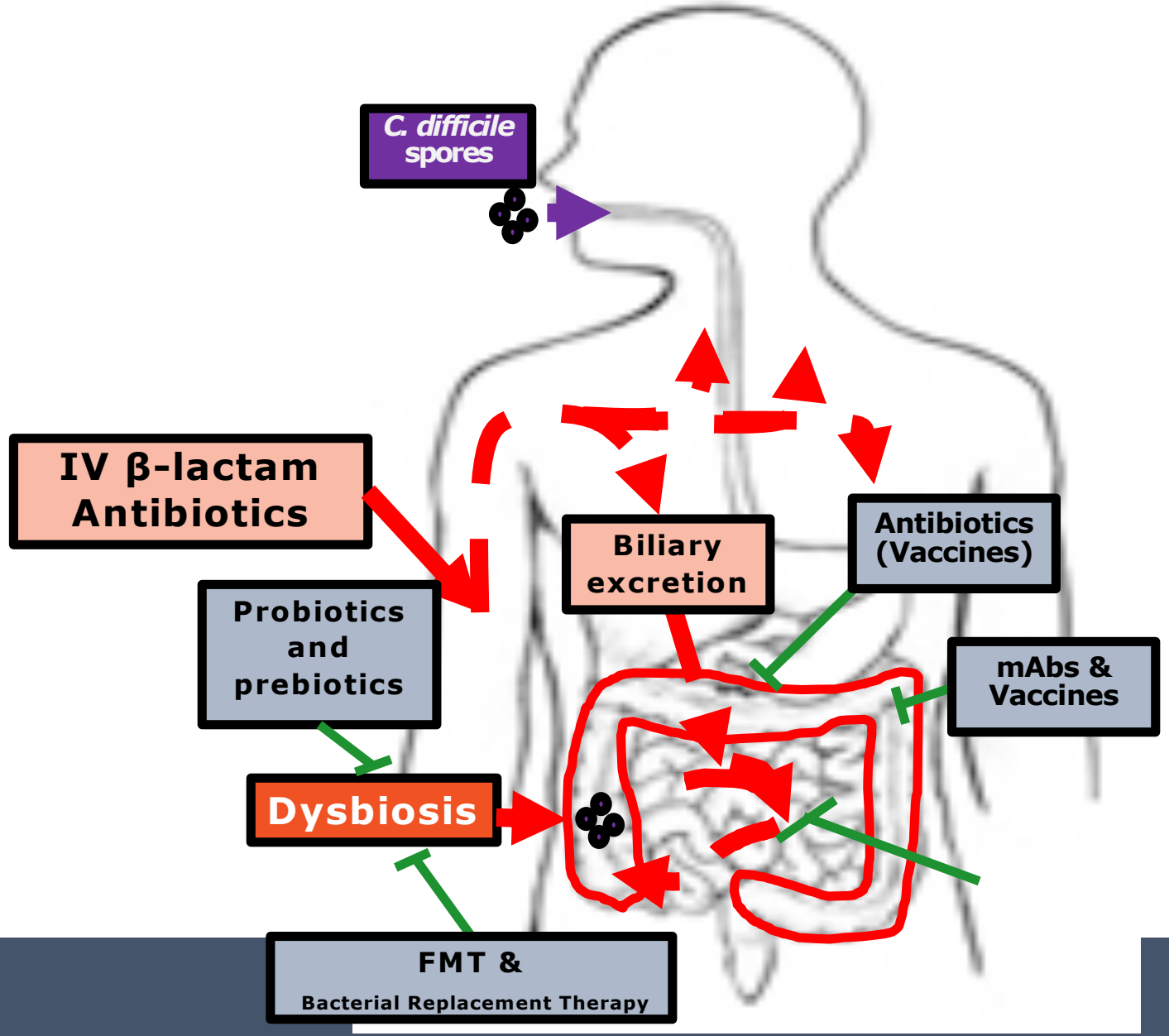

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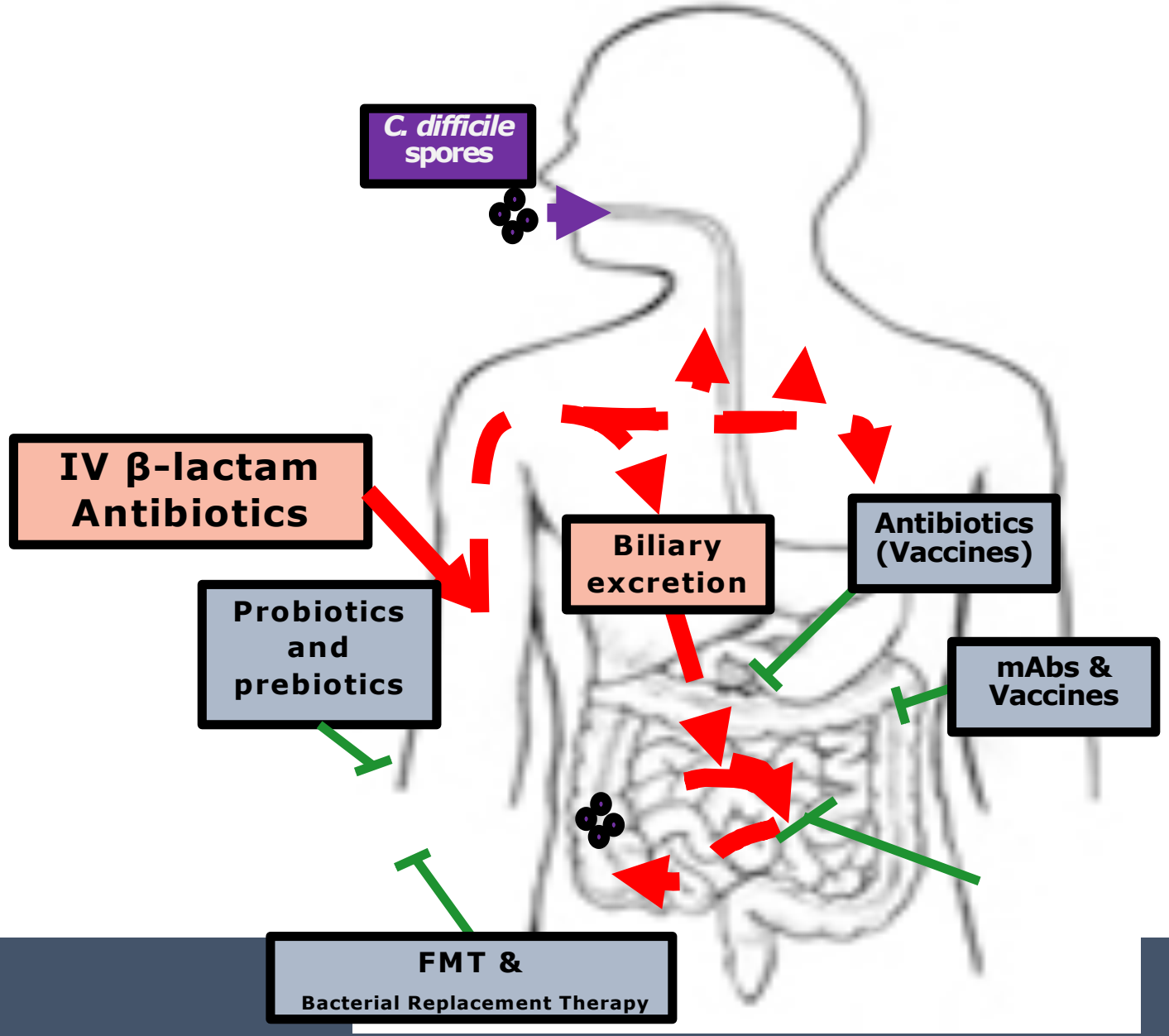

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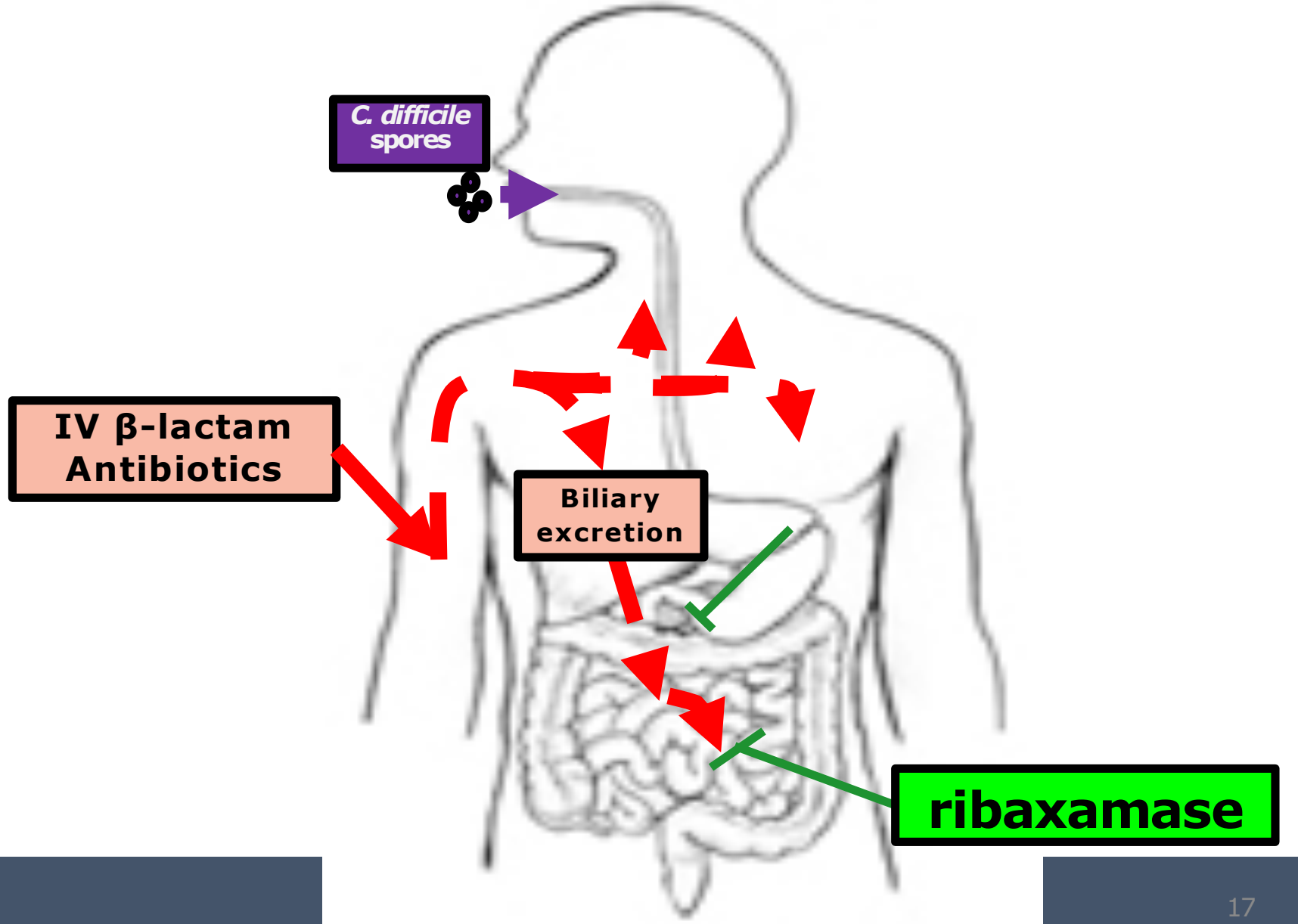

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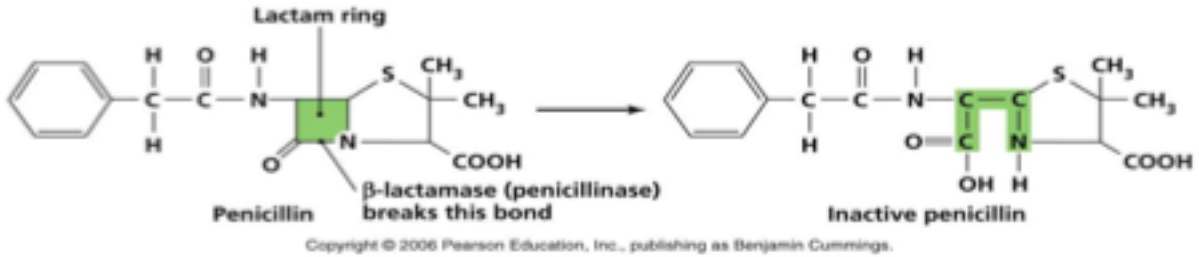
SYN-004 (ribaxamase) rye bak' sa mase

- An orally administered, β-lactamase (an enzyme of 29 kDa) that is designed to degrade penicillins and cephalosporins



- Formulated for pH-dependent release at ≥ 5.5 (proximal small intestine)
- Expected to be orally administered during and after administration of intravenous (IV) β-lactam-containing antibiotics like ceftriaxone
- Intended to degrade the excess antibiotics that are excreted into the small intestine via the bile (ribaxamase is stable in human intestinal chyme)
- Designed to prevent disruption of the gut microbiome and thus protect from opportunistic GI pathogens like *C. difficile*

Early Phase Clinical Studies

Phase 1 and Phase 2a

- **Phase 1 -** two studies in normal, healthy volunteers
  - Well tolerated up to 750 mg single dose and 300 mg q.i.d. for 7 days
  - Not systemically absorbed and no anti-drug antibodies were detected
  - Roberts et al. 2016. Clinical Drug Investigation 36: 725-734
- **Phase 2 -** two studies in subjects with functioning ileostomies, administered IV ceftriaxone ± oral ribaxamase
  - Ribaxamase degraded ceftriaxone to below the level of detection in the intestine
  - Ribaxamase did not affect the plasma PK of the ceftriaxone
  - Ribaxamase can be administered in the presence of proton pump inhibitors
  - Kokai-Kun et al. 2017. Antimicrobial Agents and Chemotherapy. 41(3):e02197-16.

Ribaxamase: Phase 2b Proof-of-Concept Study

84 Multinational Clinical Sites

Patients admitted to the hospital for treatment of a lower respiratory tract infection

Modified intent to treat = 412 patients

1:1

Ceftriaxone + Ribaxamase (***plus a macrolide***)

Ceftriaxone + Placebo (***plus a macrolide***)

Primary Endpoint:

- Prevention of *C. difficile* infection (CDI)

Exploratory Endpoints:

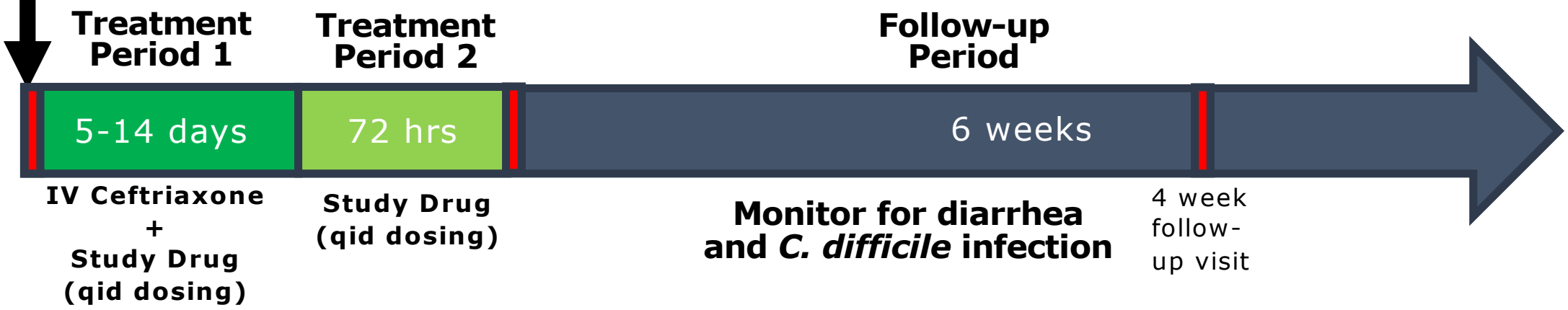
- Evaluate ability to limit disruption of the gut microbiome

Phase 2b-Proof of Concept Study

Study Design

Randomized 1:1,
150 mg ribaxamase
or placebo

US
Romania
Hungary
Serbia
Canada
Bulgaria
Poland



Fecal microbiome and fecal colonization samples taken for analysis

Diarrhea = 3 or more loose or watery stools in a 24 hour period, samples are collected
CDI = local lab results for presence of *C. difficile* toxins A and/or B by an approved test (confirmed at a central lab by toxin ELISA)

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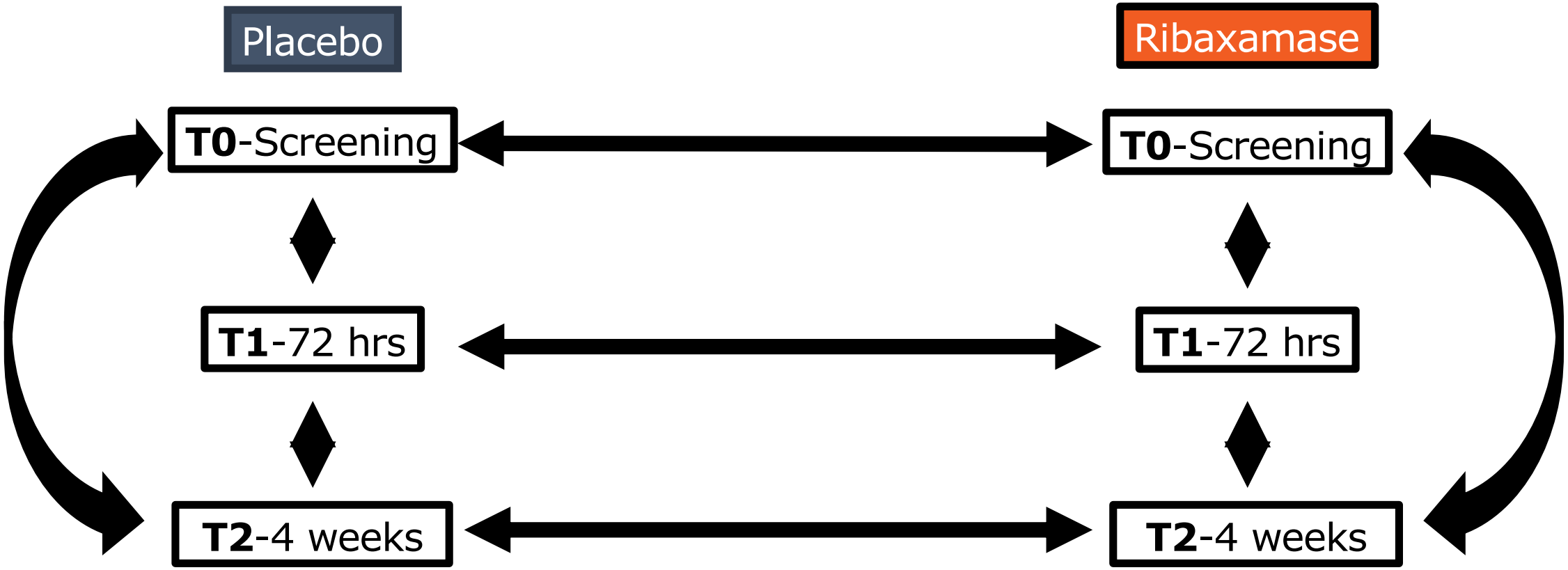
Study Demographics and Safety Outcomes

- **206** patients per group in mITT
- Average age of patients **~70** years old
- **~2/3 males** in each group
- **~1/3** of patients in each group also received **macrolides**
- **~1/3** patients received concurrent drugs for stomach acidity (**PPIs**)
- **Adverse Events**
  - Percentage of subjects reporting at least one treatment emergent adverse event (TEAE) was similar between ribaxamase and placebo groups (40.8% vs. 44.2%)
  - SAEs, including fatal AEs, were **not considered drug-related** by investigators at the clinical sites, or by an independent third-party expert, each of whom determined that SAEs were attributable to **disparities in underlying health and comorbidities** between the groups
- PI's assessment of **resolution of the LRTI was equivalent** in both groups at 2 weeks post treatment

Analysis of Changes in the Gut Microbiome

16S rRNA sequencing of DNA extracted from fecal samples



652 samples sequenced, 229 patients, 187 full-3 sample sets

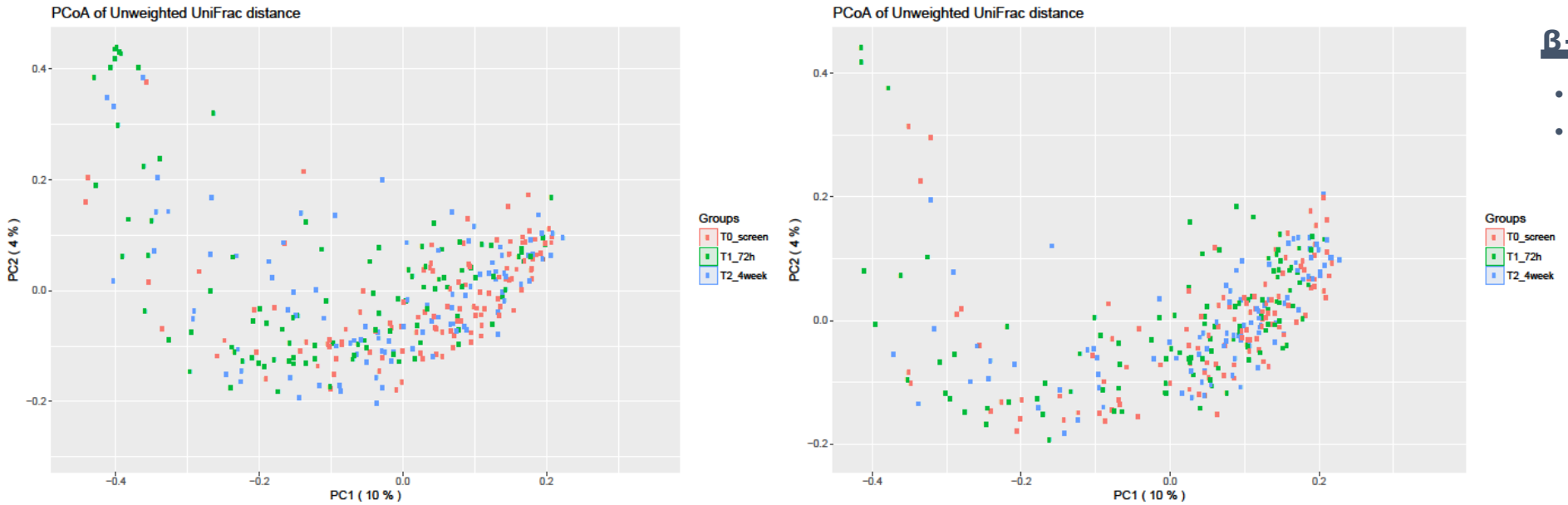
Sequencing and data analysis performed by DNA Genotek, Ottawa, Canada

SYN-004 (ribaxamase) Protected Microbial Diversity

Prevented ceftriaxone-mediated loss of **β-diversity** and enhanced microbiome recovery

Placebo **Ribaxamase**

Beta diversity compares the community composition of two different sample sets



β-diversity

- Bray-Curtis
- Unweighted Unifrac

Principle coordinate analysis of the β-diversity (unweighted Unifrac) of patient samples. β-diversity is the community composition of two different samples. Each dot represents one patient sample.

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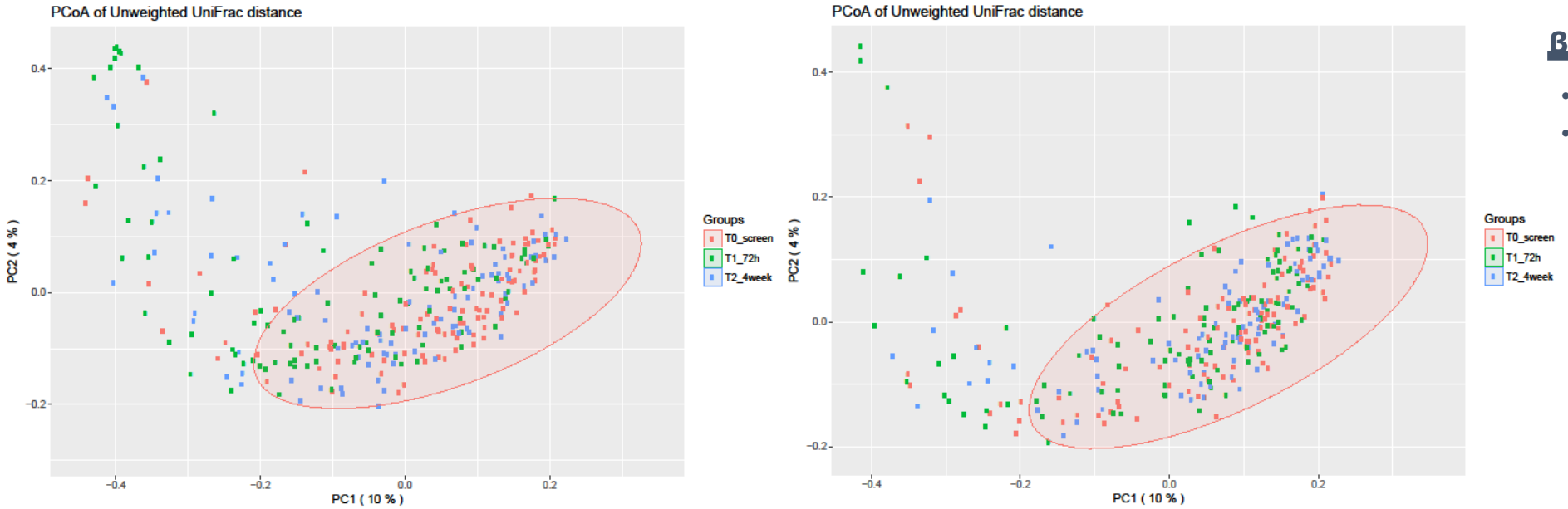
SYN-004 (ribaxamase) Protected Microbial Diversity

Prevented ceftriaxone-mediated loss of **β-diversity** and enhanced microbiome recovery

Placebo T0

Ribaxamase T0

Beta diversity compares the community composition of two different sample sets



β-diversity

- Bray-Curtis
- Unweighted Unifrac

Screening samples are similar with regards to β-diversity in both groups

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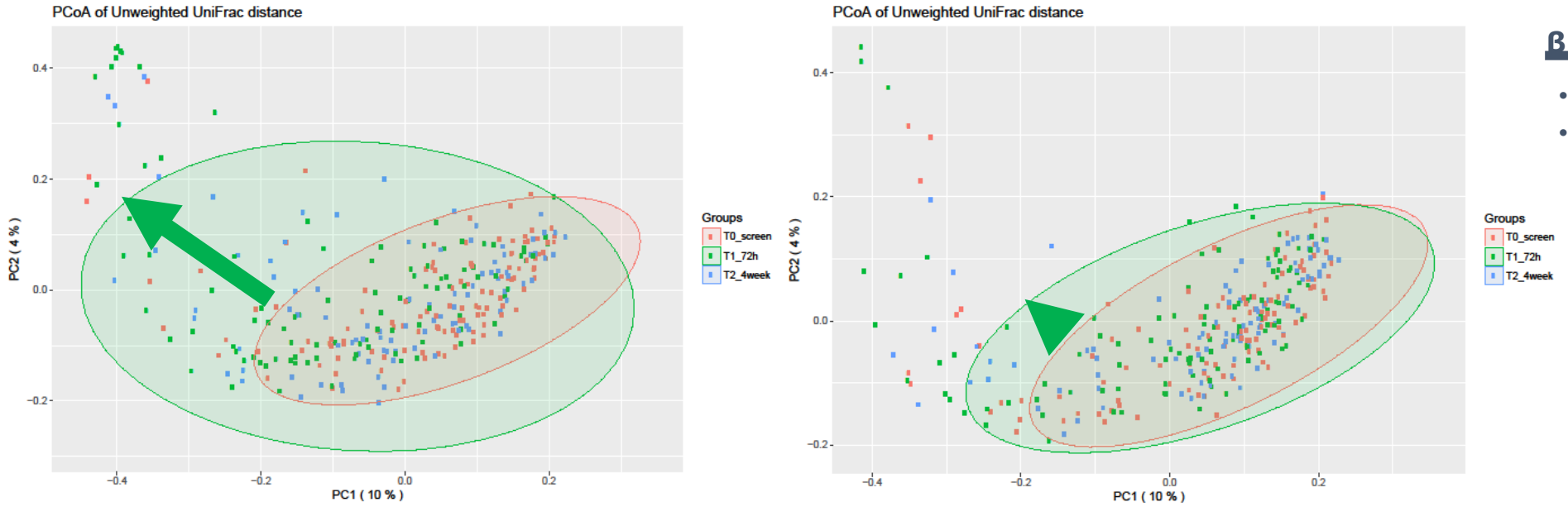
SYN-004 (ribaxamase) Protected Microbial Diversity

Prevented ceftriaxone-mediated loss of **β-diversity** and enhanced microbiome recovery

Placebo **T1** **Ribaxamase** **T1**

p=0.0025

Beta diversity compares the community composition of two different sample sets



β-diversity

- Bray-Curtis
- Unweighted Unifrac

Placebo samples display a significant loss of β-diversity as compared with ribaxamase

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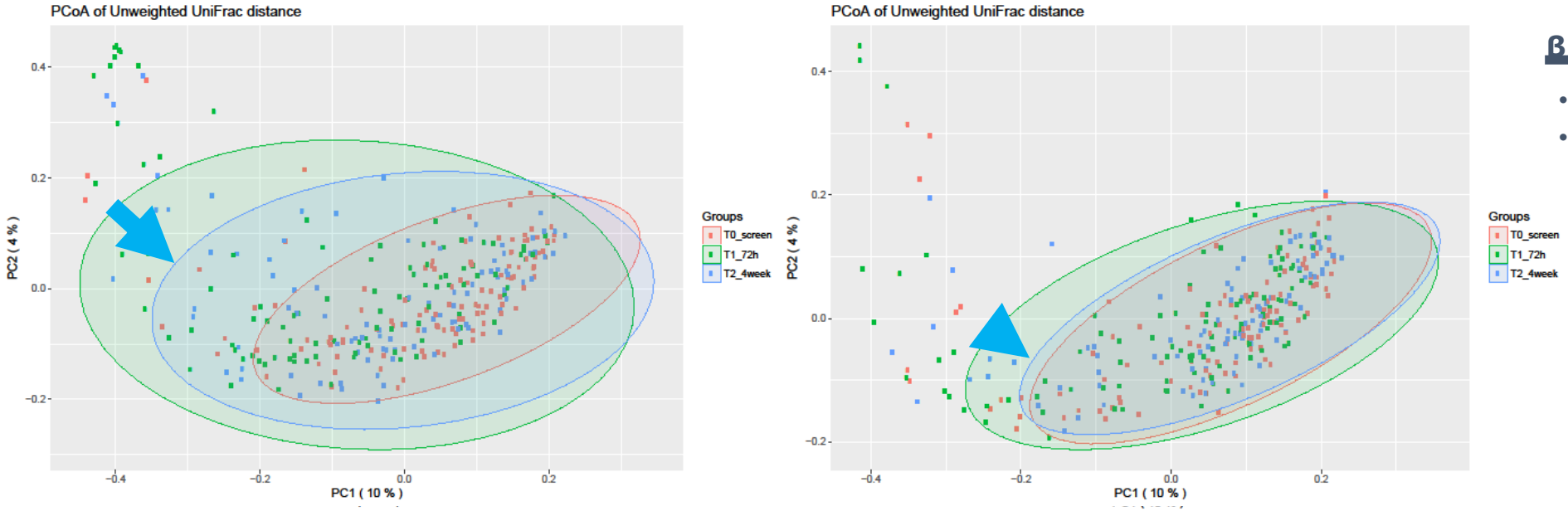
SYN-004 (ribaxamase) Protected Microbial Diversity

Prevented ceftriaxone-mediated loss of **β-diversity** and enhanced microbiome recovery

Placebo **T2** **Ribaxamase** **T2**

p=0.0064

Beta diversity compares the community composition of two different sample sets



ß-diversity

- Bray-Curtis
- Unweighted Unifrac

By T2, the ribaxamase samples have recovered to their starting diversity, but the placebo samples still display a significant loss of diversity as compared with screening

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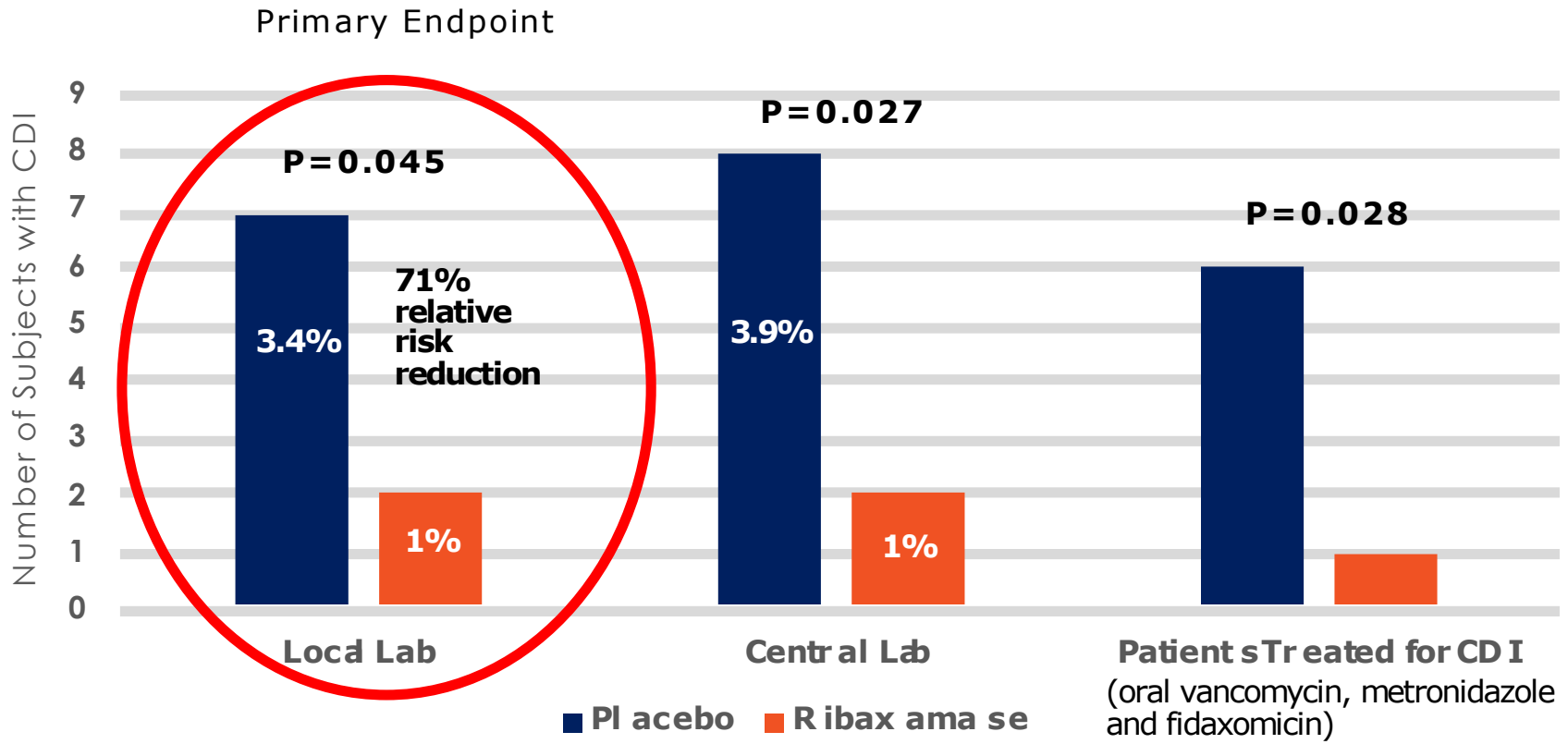
Clostridium difficile Infection (CDI)



- No CDI patients reported previous CDI
- P-values are 1-sided based on the pre-specified Z-test
- The study was powered at 80% with 1-sided alpha=0.05

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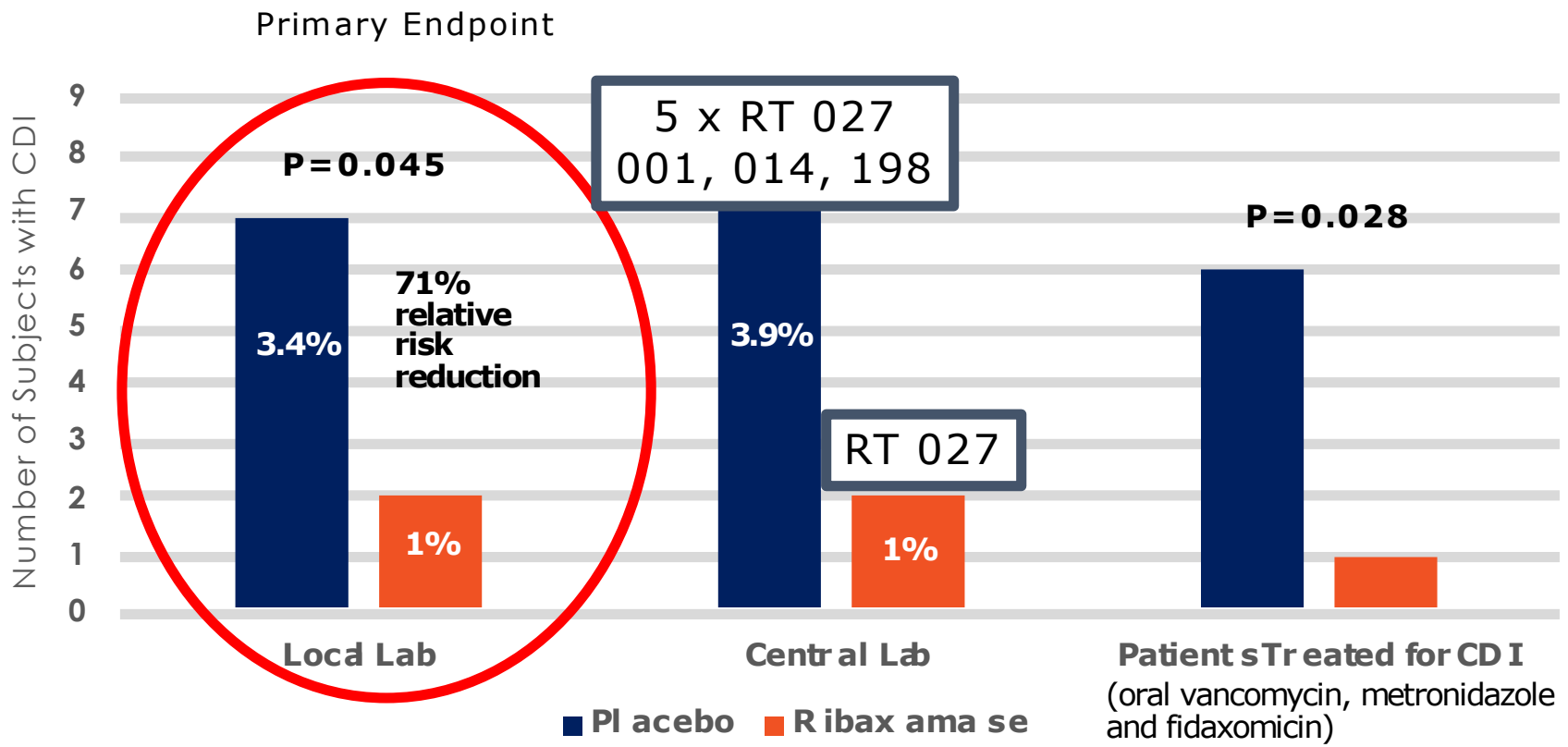

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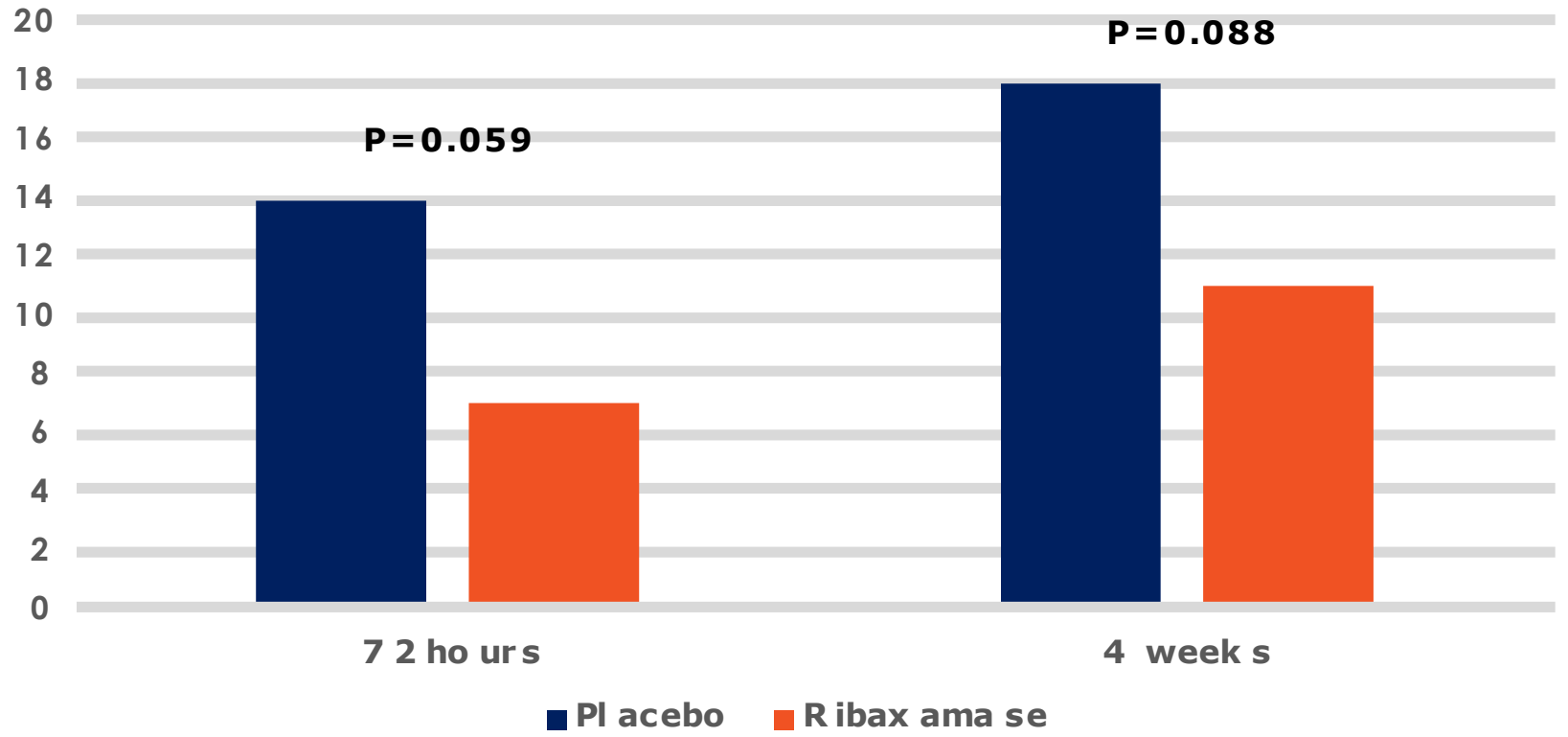
New *C. difficile* Colonization at 72 hrs & 4 weeks



- New colonization is negative on screening and then positive on a subsequent sample
- P-values are 1-sided based on the pre-specified Z-test

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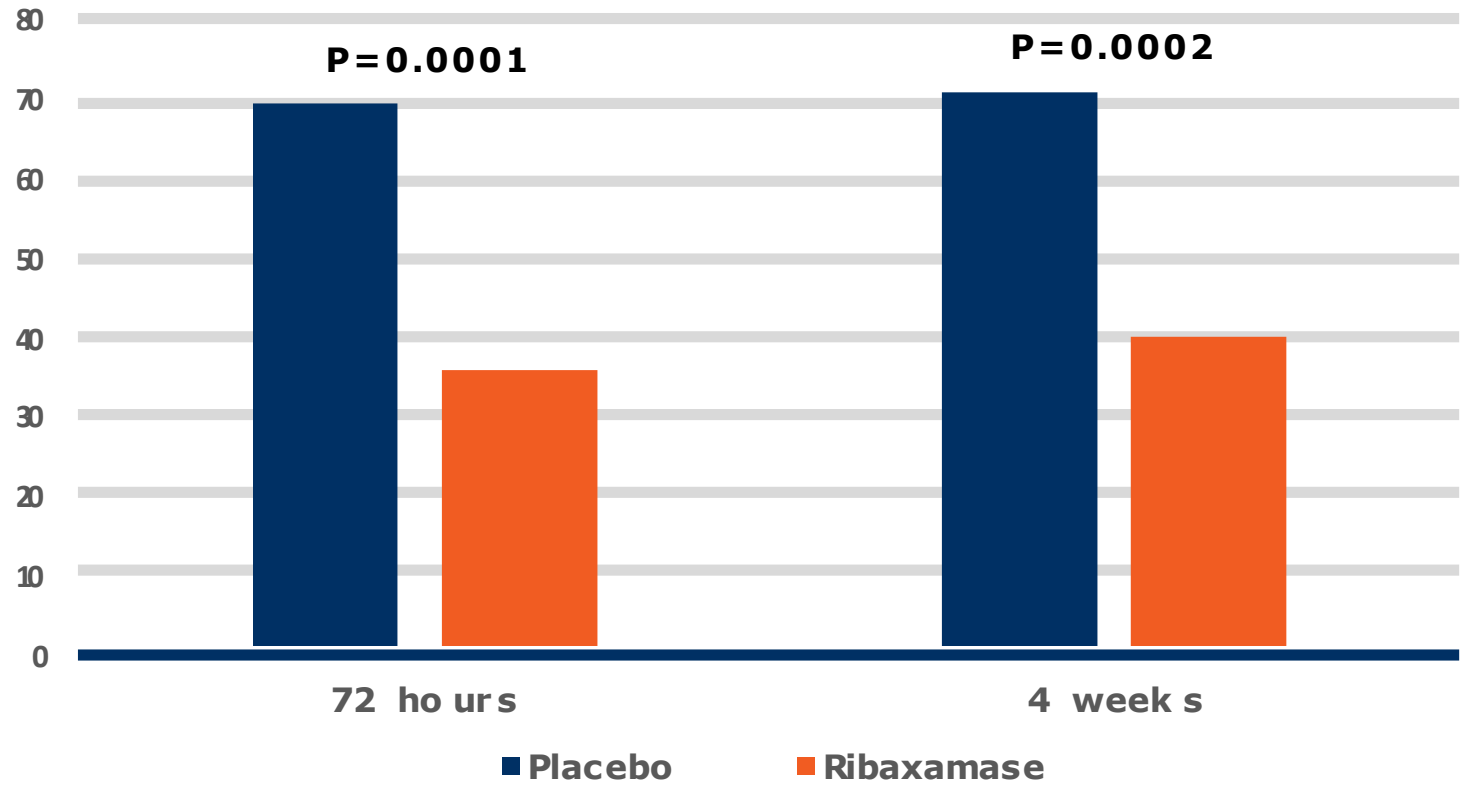
New VRE Colonization at 72 hrs & 4 weeks



- P-values are 1-sided based on the pre-specified Z-test

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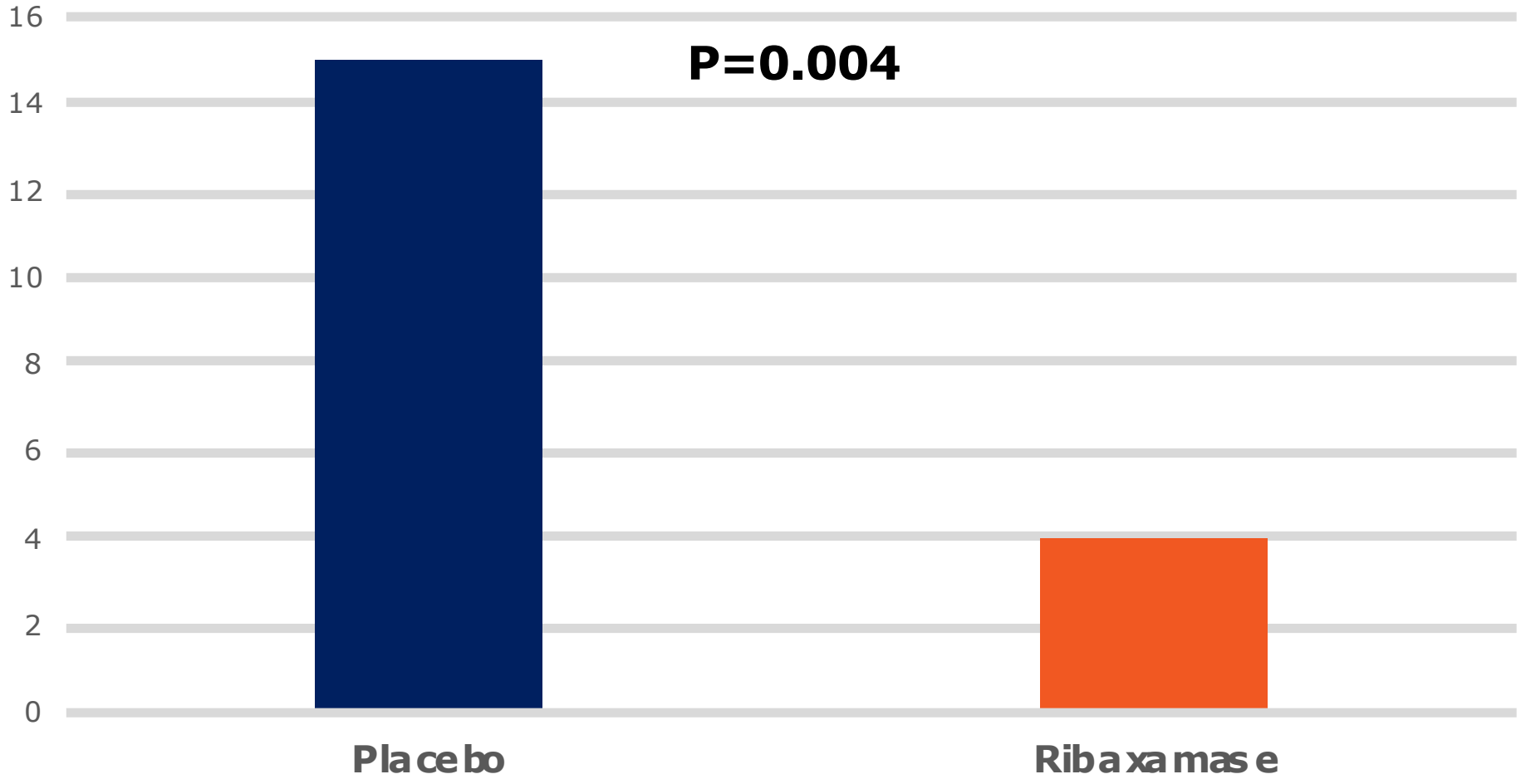
Comparison of Patients with Enterococcal Mono-domination

> 30% of taxa present were enterococci at T1 or T2 based on 16S sequencing



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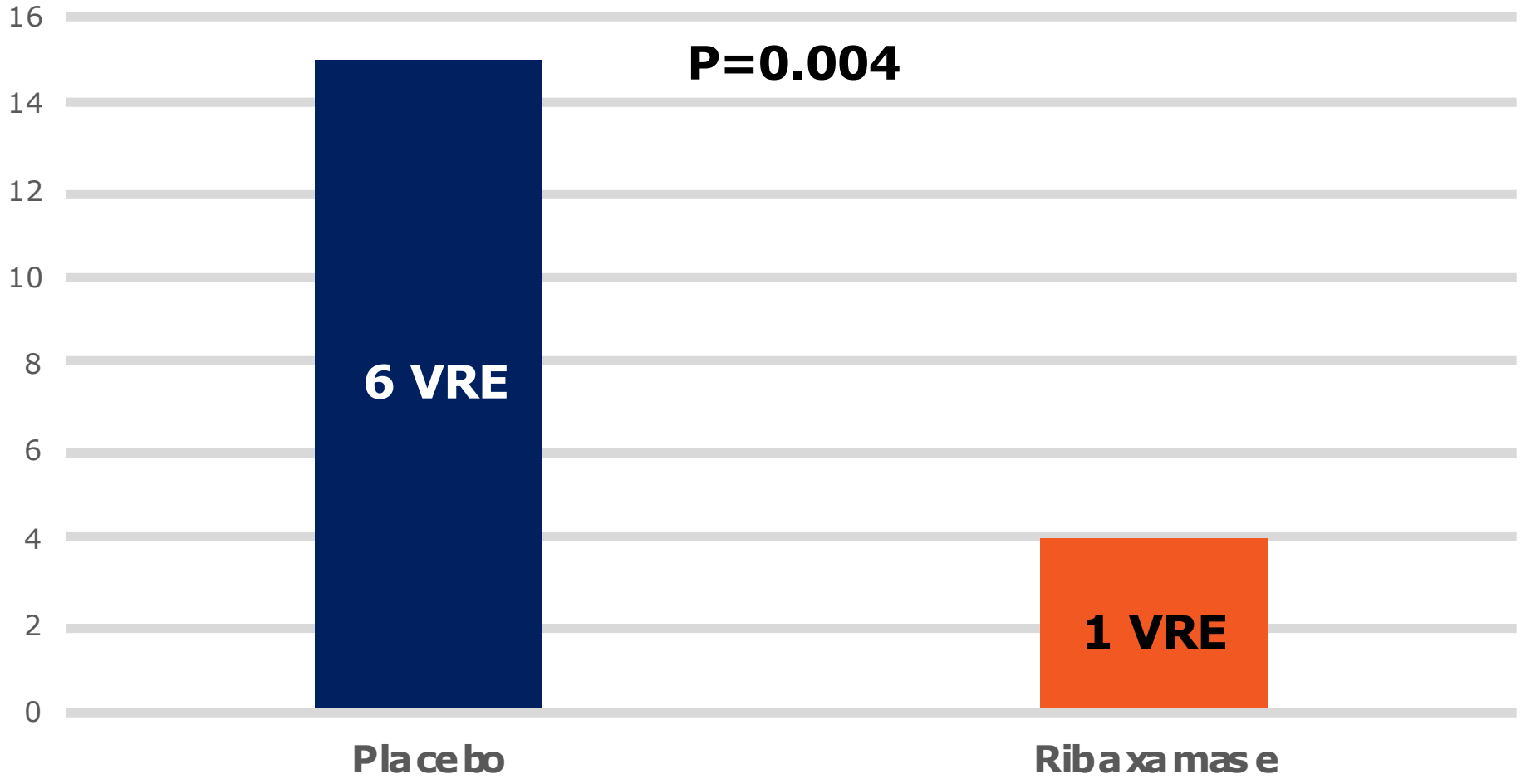

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Resistome Analysis of Longitudinal Fecal Samples

- DNA extracted from **350 fecal samples** sequenced by whole genome shotgun sequencing (Diversigen, Houston, TX)
- Interrogated against the **CARD database**
- 21,000,000 DNA matches
- **1300 AMR genes** identified with ~60,000 matches per sample
- Total hits per AMR gene ranged from 1 to 2.3M (*tetQ*, *tetW*)
- Including many genes of interest, **β-lactamases, vancomycin and macrolide resistance** genes
- Statistical analysis was performed to determine which genes significantly changed from the screening sample (T0) to the post antibiotic sample (T1) in the placebo vs. the ribaxamase patients

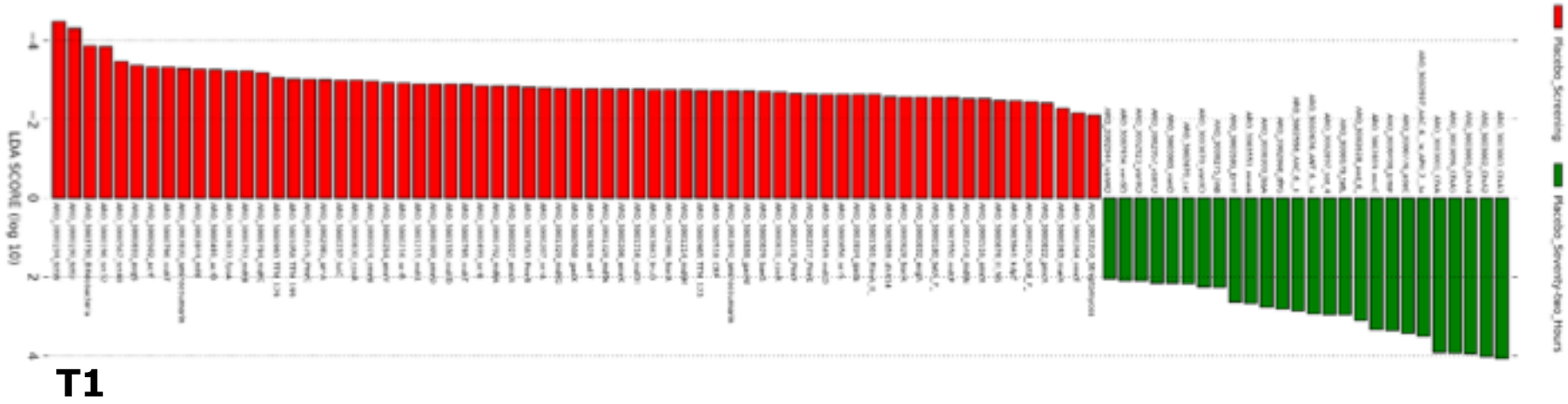
Analysis of the Change in Relative Abundance of AMR Genes

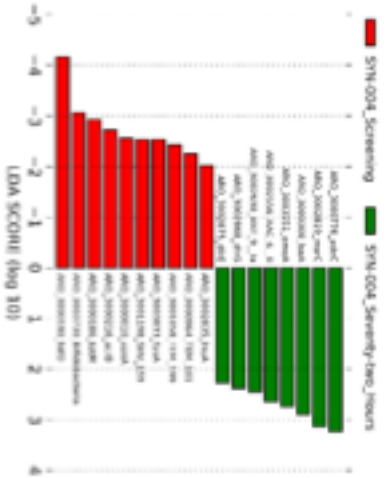
Collection point T0 to T1, Placebo vs. Ribaxamase-treated patients

T0

Placebo

Ribaxamase





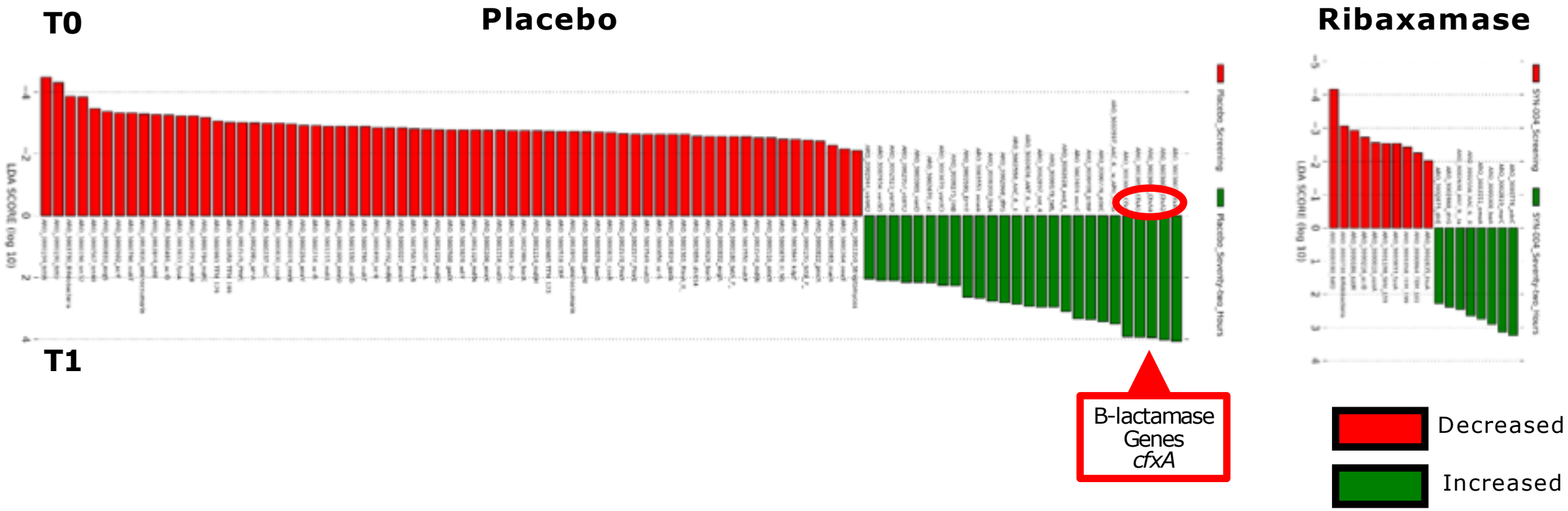
T1

Decreased

Increased

Analysis of the Change in Relative Abundance of AMR Genes

Collection point T0 to T1, Placebo vs. Ribaxamase-treated patients



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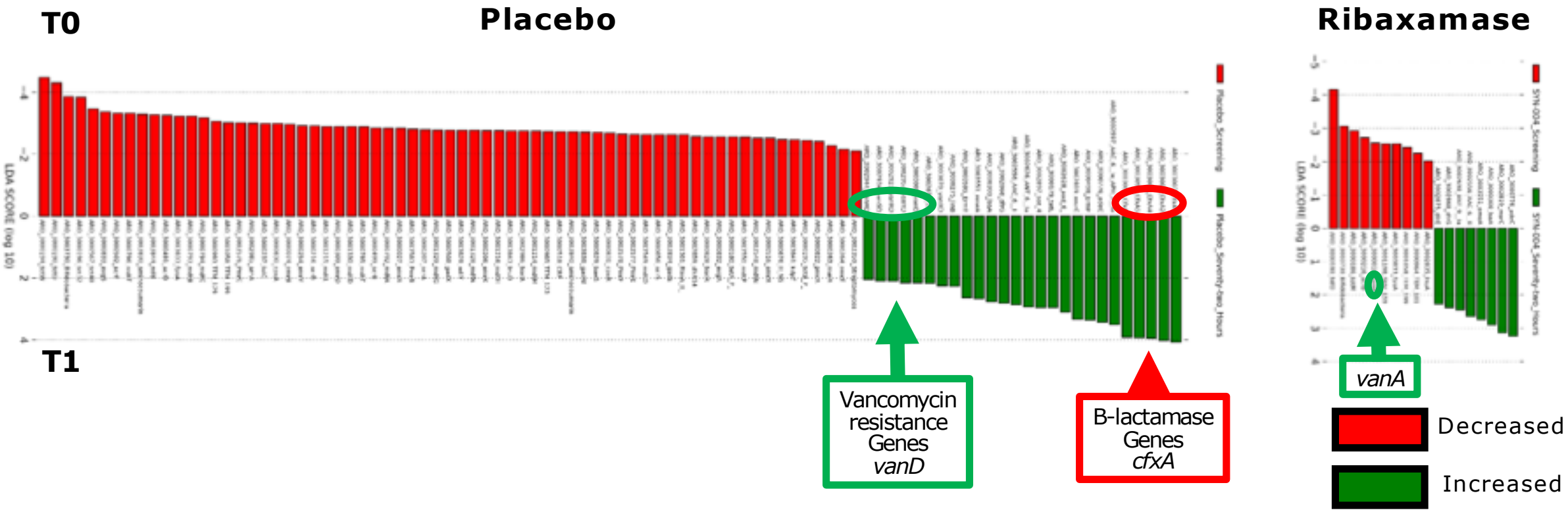

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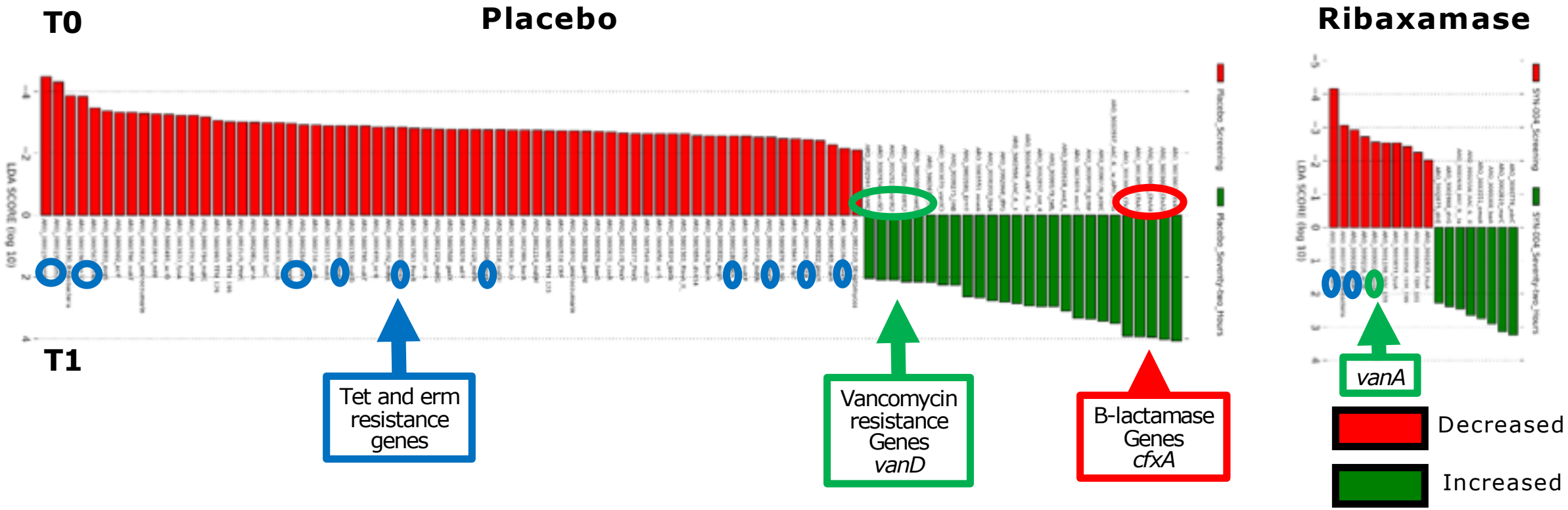

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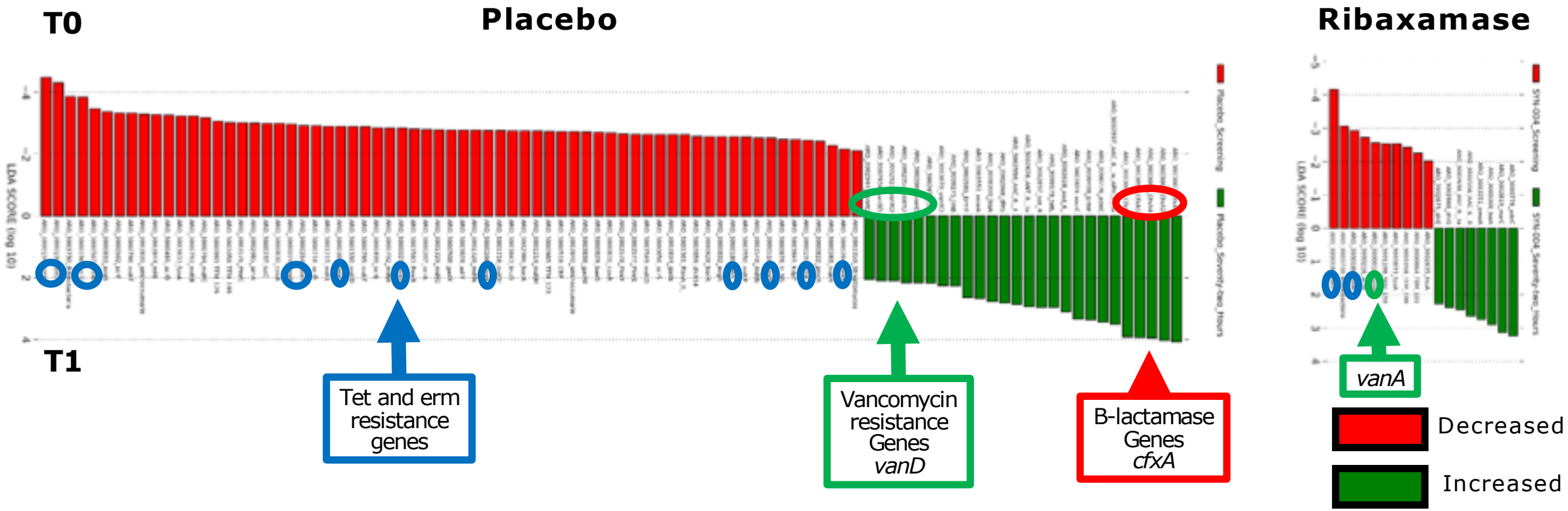


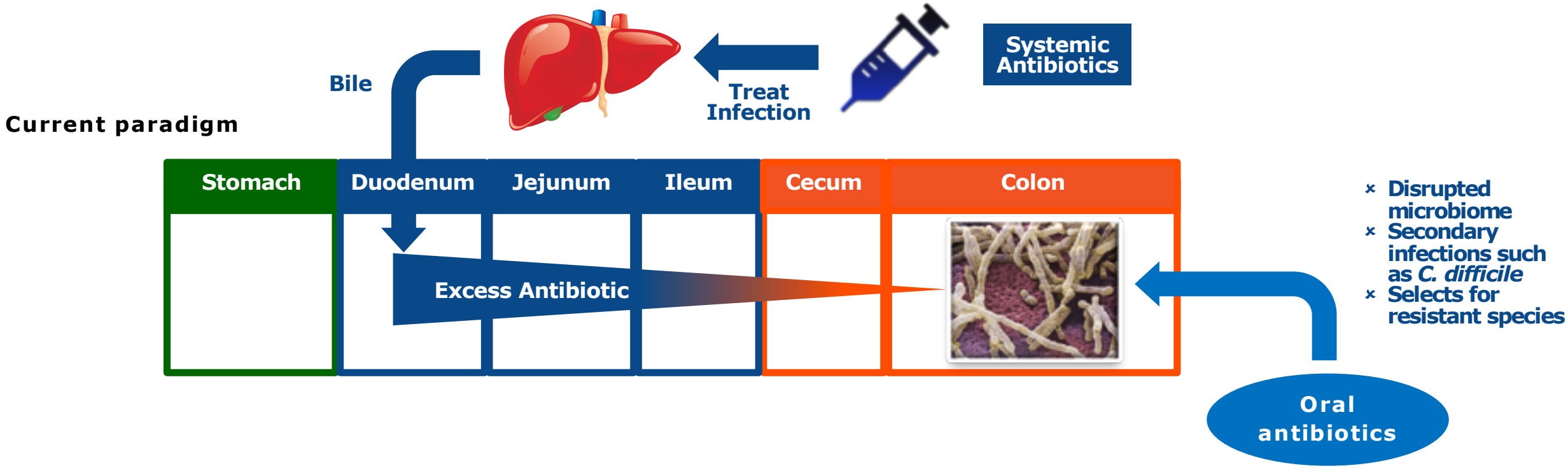
Confirmed by qPCR analysis of *cfxA1* and *vanRD*

Conclusions

- Ribaxamase **reduced the incidence** of new onset CDI by 71% as compared with placebo (confirmed at the central lab), p=0.045
- Ribaxamase **protected the diversity** of the gut microbiome
- Ribaxamase appeared to be **well-tolerated** and **not affect the cure rate** for the primary infection
- Ribaxamase **reduced new colonization** with *C. difficile* and VRE, (p=0.0002), reduced enterococcal mono-domination
- Ribaxamase **reduced ceftriaxone-induced changes** in the gut resistome which could result in a general reduction of AMR

Ribaxamase Represents a Paradigm Shift

In the Use of Intravenous β-lactam Antibiotics



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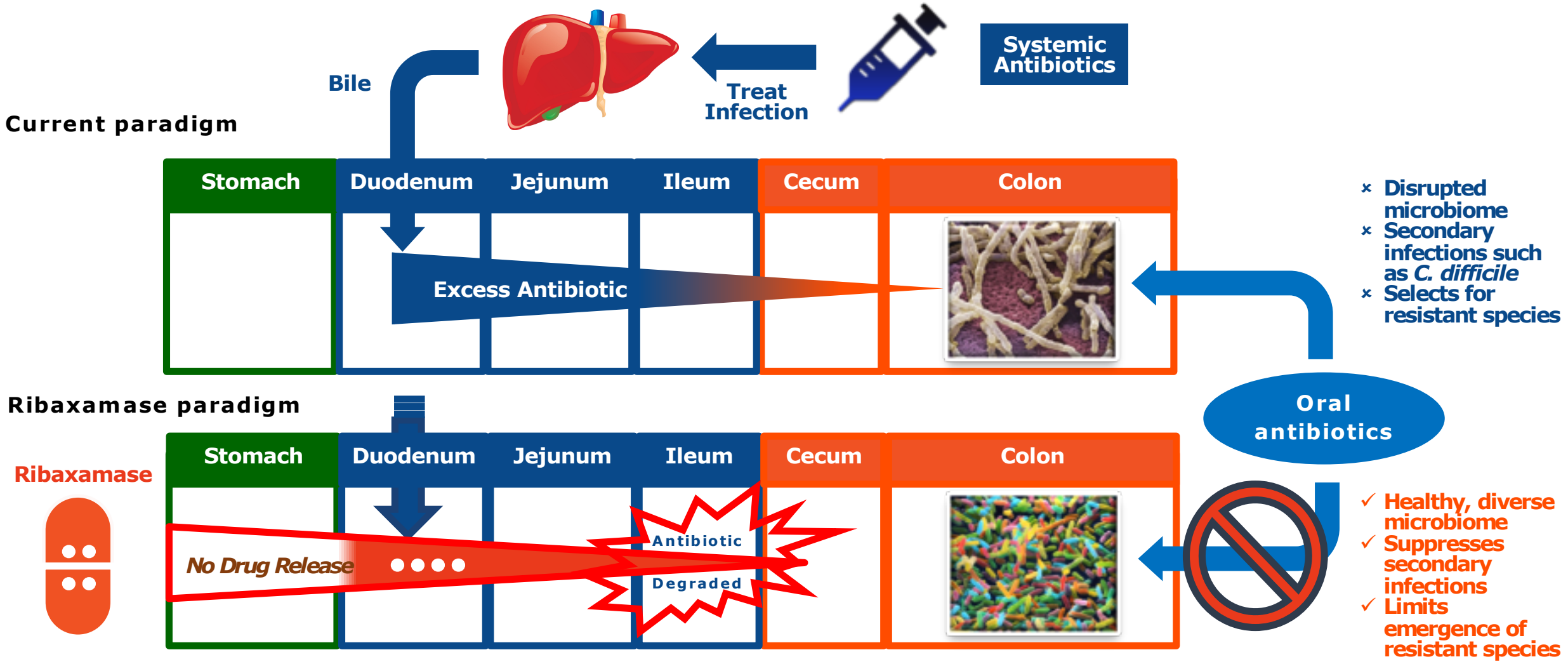
Ribaxamase Represents a Paradigm Shift

In the Use of Intravenous β-lactam Antibiotics



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Questions?