

# Gut Microbiome in GWI, An unstable Resistome and Targeted Therapeutics

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**Meeting of the Research Advisory Committee on Gulf War Veterans' Illness  
March 10<sup>th</sup>, 2021**

**VA**



U.S. Department  
of Veterans Affairs



UNIVERSITY OF  
**SOUTH  
CAROLINA**

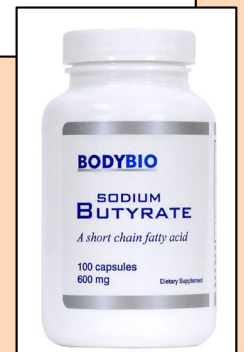
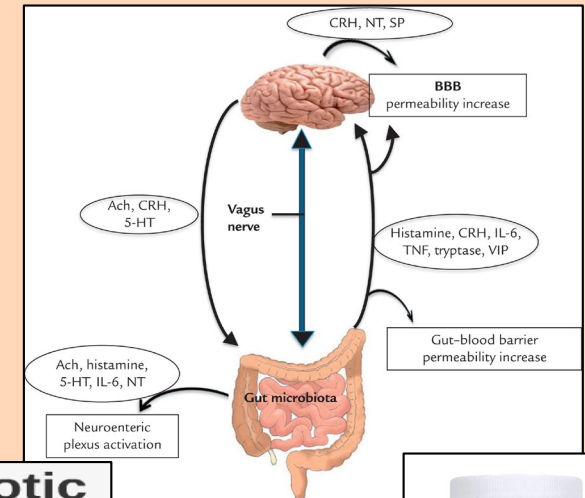


## 1. Altered gut microbiome and virome and the Gut-Brain-Axis (pathology) and Gut-Liver Axis (pathology).

## 2. Drug Discovery: Gut microbiome targeted therapeutics.

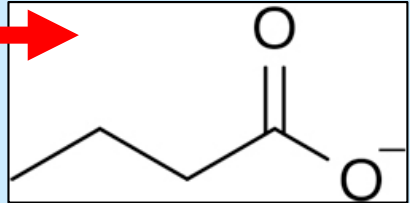
## 3. Modelling susceptibility to disease in underlying GWI:

- Rising to future challenges.
- Obesity.
- Infectious Disease.
- Metabolic Syndrome.
- Mechanisms of Susceptibility (Resistome).

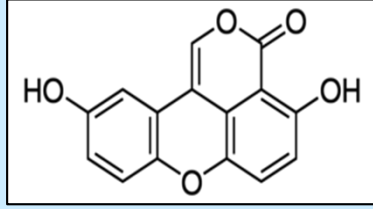


1. Targeting the Altered gut microbiome
2. Targeting the Gut-Brain-Axis (pathology) and Gut-Liver Axis (pathology).
3. Targeting the Virome
4. Targeting the Altered Resistome

**Butyrate**



**Sparstolonin B**



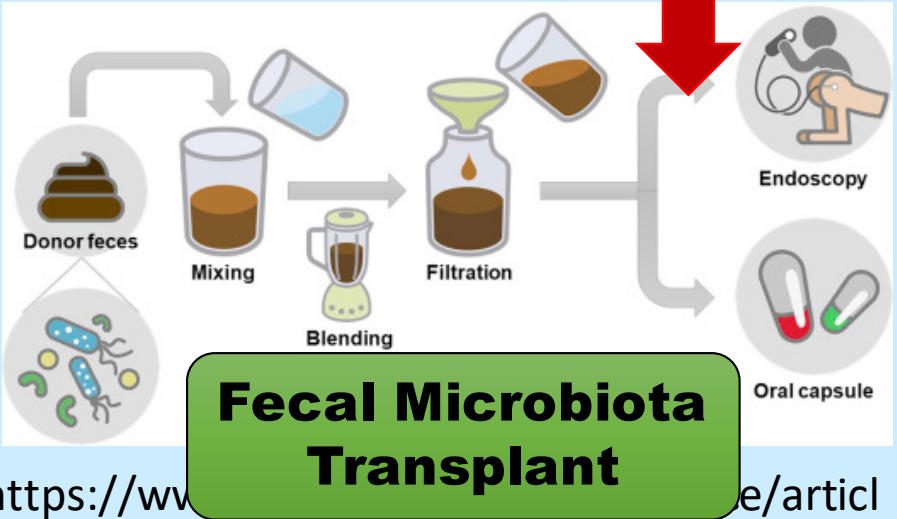
brain sciences

Open Access Article

**TLR Antagonism by Sparstolonin B Alters Microbial Signature and Modulates Gastrointestinal and Neuronal Inflammation in Gulf War Illness Preclinical Model**

by Dipro Bose<sup>1</sup>, Ayan Mondal<sup>1</sup>, Punng Saha<sup>1</sup>, Diana Kimono<sup>1</sup>, Sutapa Sarkar<sup>1,†</sup>, Ratanes K. Seth<sup>1</sup>, Patricia Janulewicz<sup>2</sup>, Kimberly Sullivan<sup>2</sup>, Ronnie Horner<sup>3</sup>, Nancy Klimas<sup>4,5</sup>, Mitzi Nagarkatti<sup>6</sup>, Prakash Nagarkatti<sup>6</sup> and Saurabh Chatterjee<sup>1,7,\*</sup>

<sup>1</sup> Environmental Health and Disease Laboratory, Department of Environmental Health Sciences, University of South Carolina, Columbia, SC 29208, USA

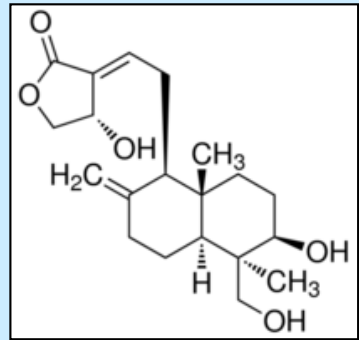


**PROBIOTICS**

LACTOBACILLUS LACTOCOCCUS PROPIONIBACTERIUM  
STREPTOCOCCUS THERMOPHILUS BIFIDOBACTERIUM BULGARICUS

**Manuscript in progress.....**

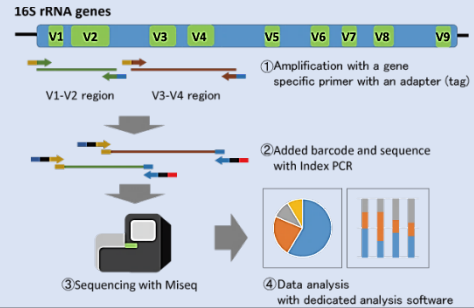
**Andrographolide**



**Manuscript in progress.....**

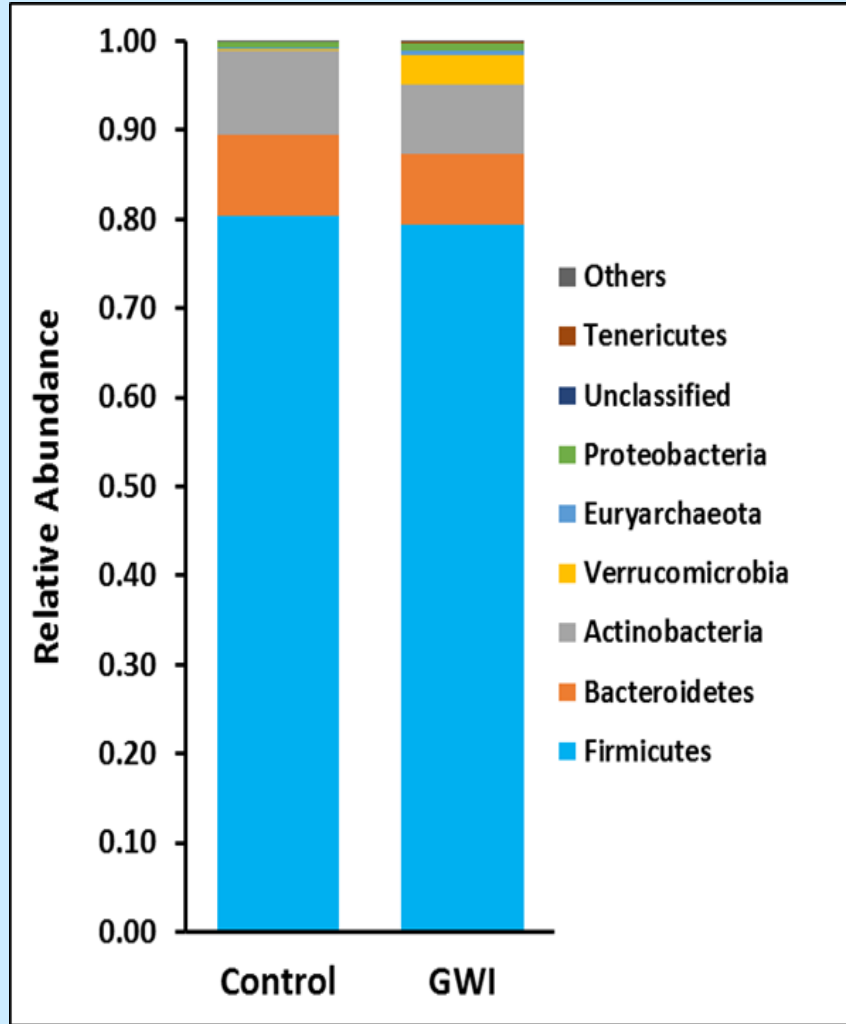
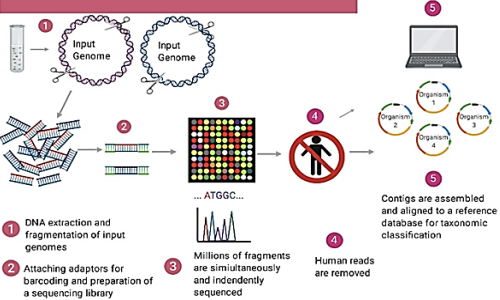
## Gut Bacteriome in GWI veterans (GWIC/BBRAIN cohorts)

### From 16S Sequencing

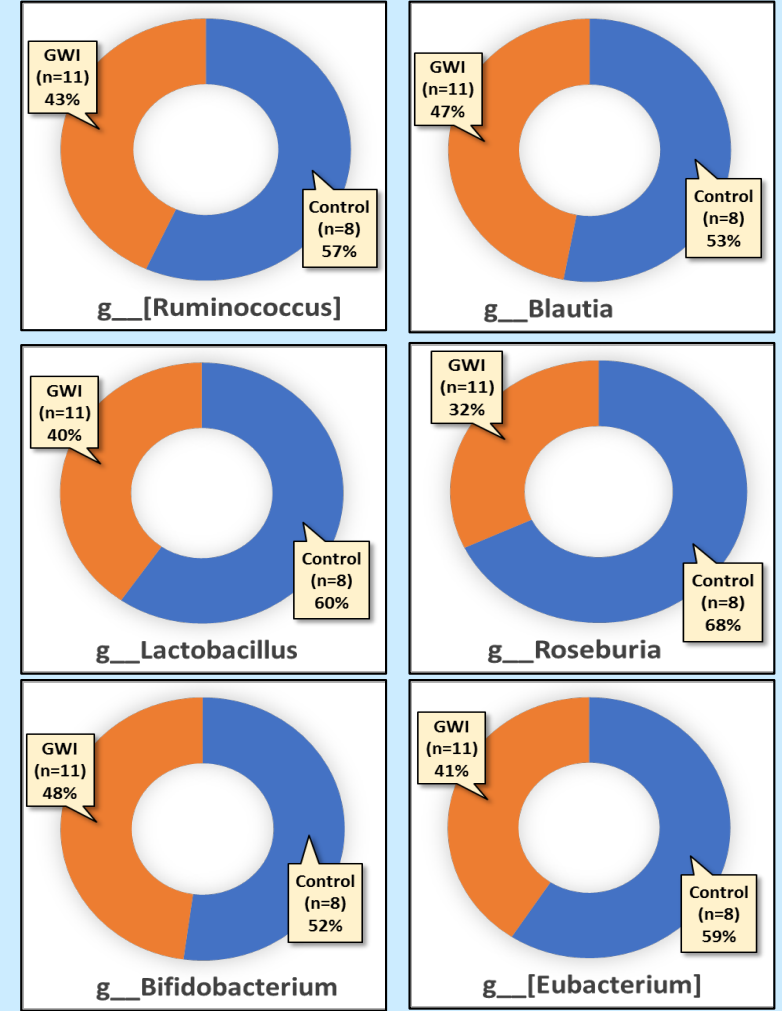


### To NGS & Metagenomics

#### Metagenomic Next-Generation Sequencing



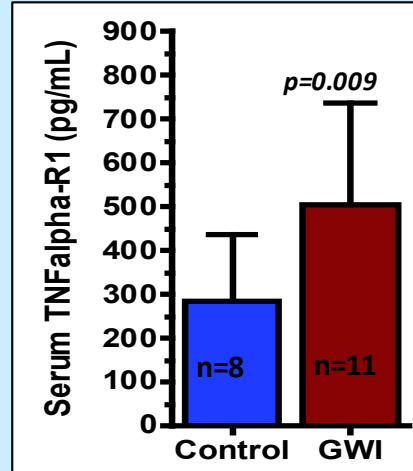
Relative abundances of bacterial phyla.



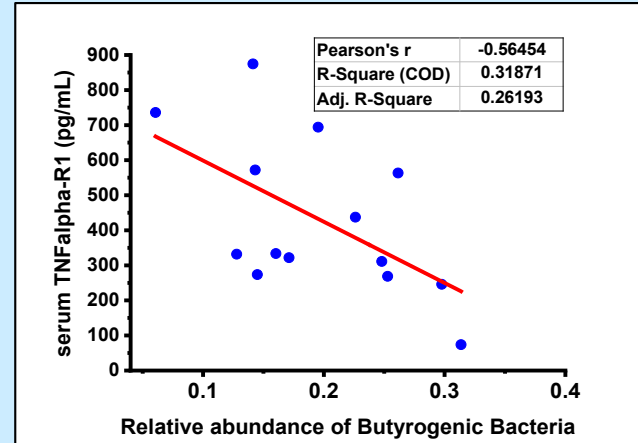
Relative abundances of bacterial species.

## Role of sTNF $\alpha$ -R1 in various pathophysiology:

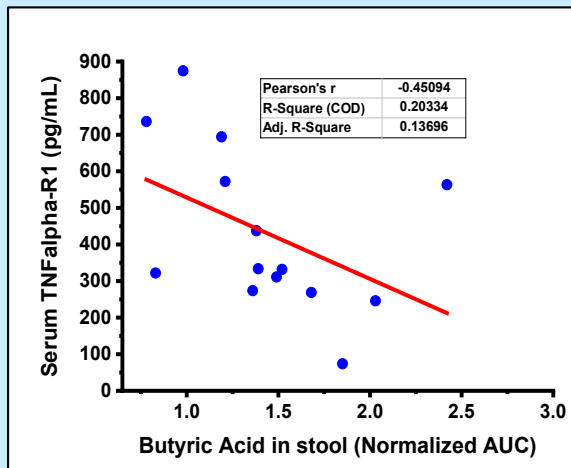
- Interaction of TNF $\alpha$  with sTNF $\alpha$ -R1 leads to hepatic stellate cell activation, leading to liver fibrosis.
- Targeted therapy against sTNF $\alpha$ -R1 signaling slows neuronal loss and amyloid beta generation in a mouse model of Alzheimer's disease.
- Increased circulatory level of sTNF $\alpha$ -R1 is often associated with various kidney diseases and disease progression.



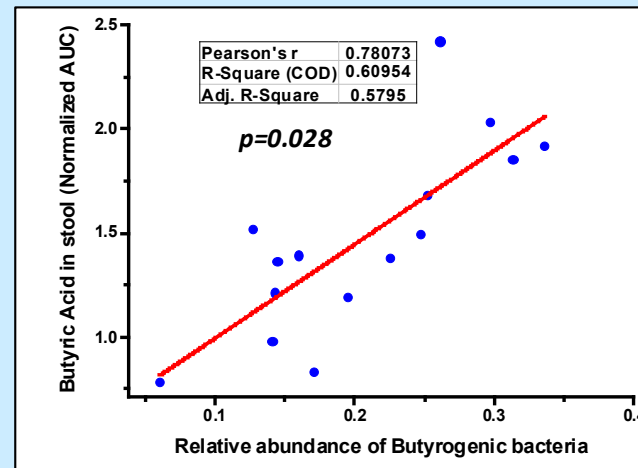
Serum level of TNF $\alpha$ -R1



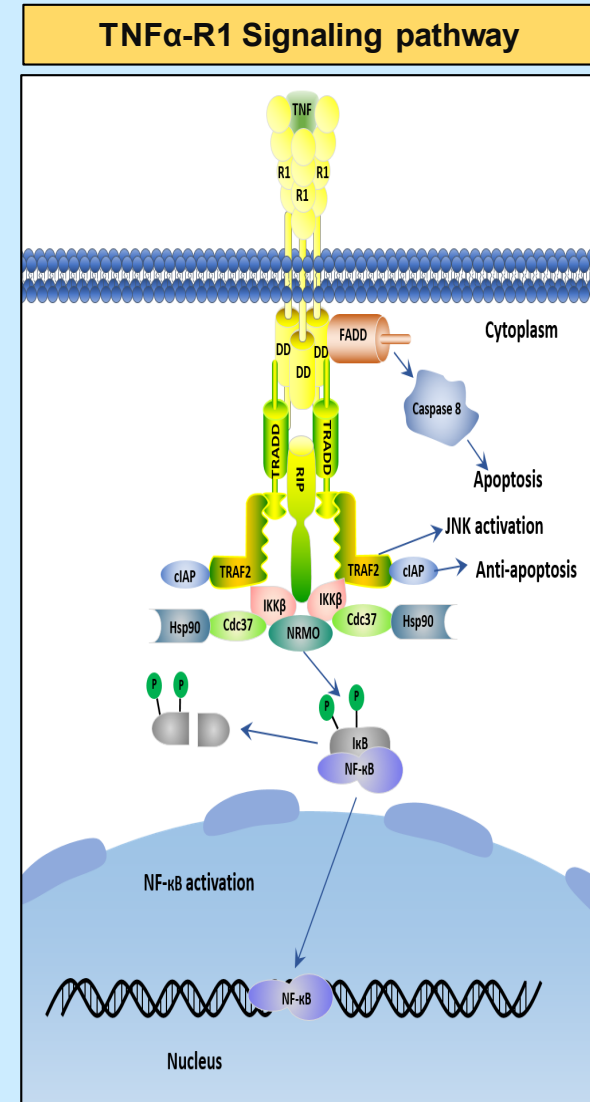
Association of serum TNF $\alpha$ -R1 with butyrogenic bacteria



Correlation of serum TNF $\alpha$ -R1 with butyric acid in stool

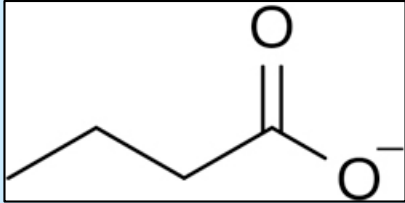


Association of fecal TNF $\alpha$ -R1 with butyrogenic bacteria



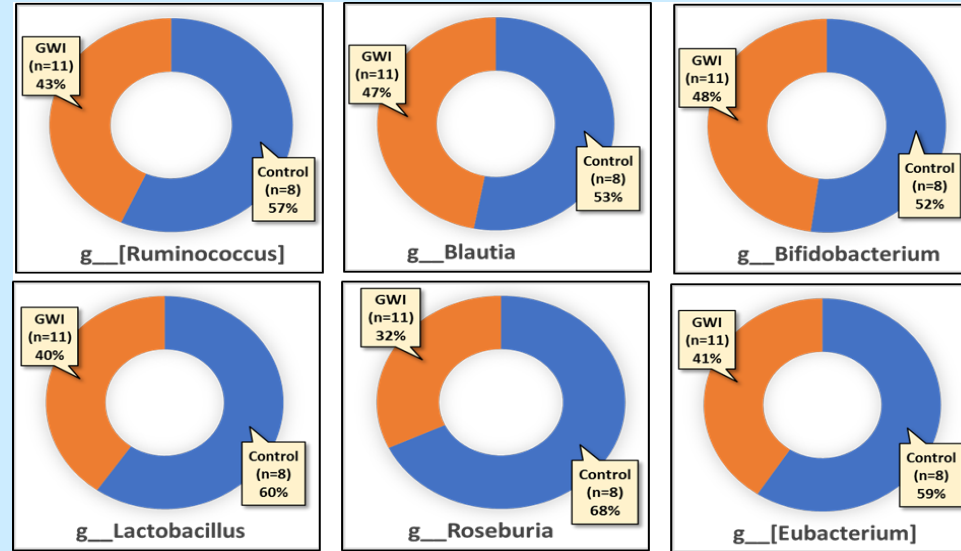


### Butyrate

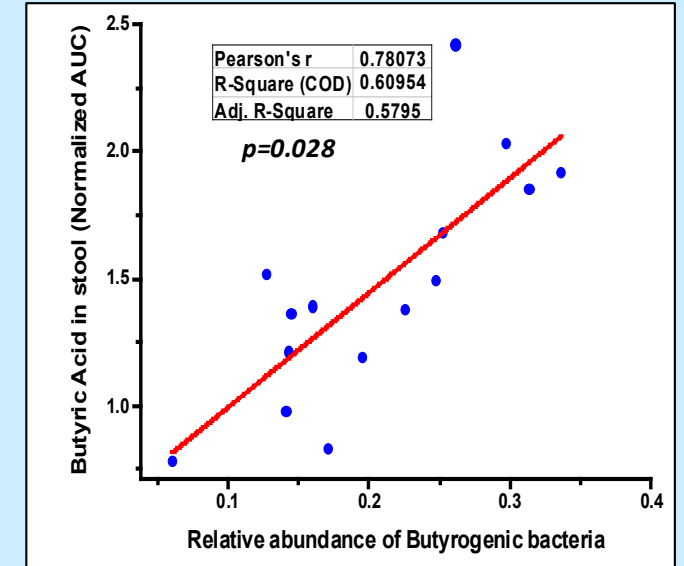


### Established Roles in Clinical Trials

- Intestinal Inflammation
- Traveller's Diarrhoea
- Irritable Bowel Syndrome
- Ulcerative colitis
- Obesity
- Neurocognition



Butyrogenic Bacteria in GWI Veterans



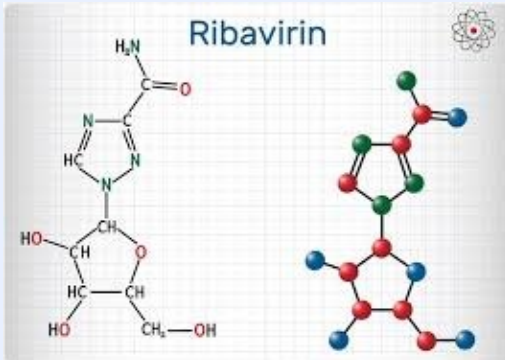
Butyrate level in GWI veterans

- ❖ 18 weeks clinical trial.
- ❖ Primary outcome VSF36 & CVLT-II.
- ❖ Secondary - Fatigue/Pain/Bowel Symptom Score.
- ❖ Mechanistic - Intestinal Permeability.
  - Bacteriome – Virome Analysis.
  - CNS auto antibodies and its relationship with gut bacteriome/virome

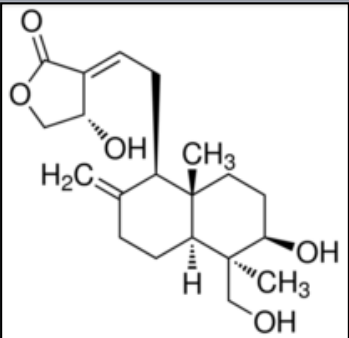
# Targeted Therapeutics for Virome in Gulf War Veterans

## Chatterjee Lab findings

### Gut Bacteriome in GWI veterans (GWIC/BBRAIN cohorts)



### Andrographolide



**Ribavirin: FDA approved antiviral formulation**  
Widely used since 1990s  
Broad spectrum effects  
Safety issues persist that include hemolysis

Phase-1 clinical trial in GWI veterans can be proposed with Ribavirin and Vitamin E as a combination therapy

**Repurposed Therapy**



**Andrographolide: Not FDA approved antiviral, analgesic, antipyretic, formulation**

Widely used since centuries  
Broad spectrum effects  
No toxicity data in clinical trials  
FDA IND required  
Phase-1 clinical trial in GWI veterans can be proposed with Andrographolide

**Phase-I study required**



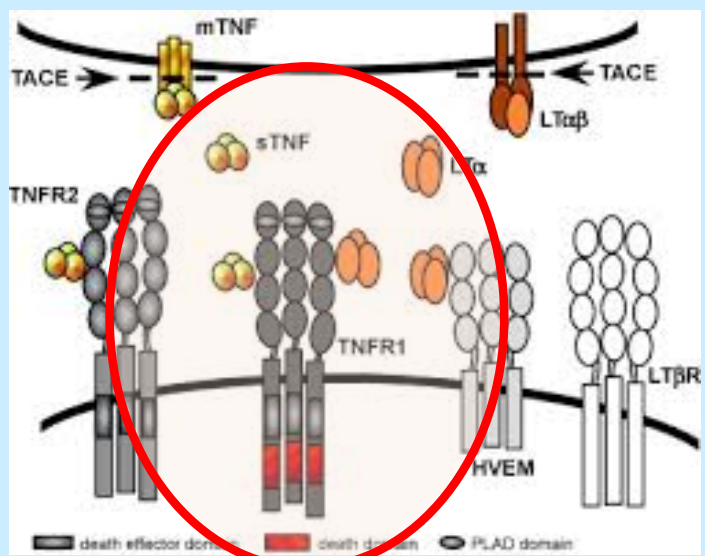
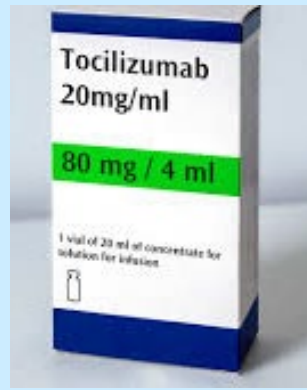
# Role of TNFR1 and IL6 in Gulf War Illness and possible drug candidates (Chatterjee Lab)

Chatterjee Lab clinical and preclinical data

**Infliximab**



**Tocilizumab**



**IL-6**

International Journal of Environmental Research and Public Health

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- Edit a Special Issue

Article Menu

Open Access Article

## The Gut-Microbiome in Gulf War Veterans: A Preliminary Report

by Patricia A. Janulewicz<sup>1,\*</sup>, Ratanesh K. Seth<sup>2</sup>, Jeffrey M. Carlson<sup>1</sup>, Joy Ajama<sup>1</sup>, Emily Quinn<sup>3</sup>, Timothy Heeren<sup>4</sup>, Nancy Klimas<sup>5</sup>, Steven M. Lasley<sup>6</sup>, Ronnie D. Horner<sup>7</sup>, Kimberly Sullivan<sup>1,†</sup> and Saurabh Chatterjee<sup>2,†</sup>

**TNFR1 is associated with GWI in veterans**

viruses

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Article Menu

Article Overview

Open Access Article

## Gut DNA Virome Diversity and Its Association with Host Bacteria Regulate Inflammatory Phenotype and Neuronal Immunotoxicity in Experimental Gulf War Illness

by Ratanesh K. Seth<sup>1</sup>, Rabia Maqsood<sup>2</sup>, Ayan Mondal<sup>1</sup>, Dipro Bose<sup>1</sup>, Diana Kimono<sup>1</sup>, LaRinda A. Holland<sup>2</sup>, Patricia Janulewicz Lloyd<sup>3</sup>, Nancy Klimas<sup>4</sup>, Ronnie D. Horner<sup>5</sup>, Kimberly Sullivan<sup>3</sup>, Efrem S. Lim<sup>2,\*</sup> and Saurabh Chatterjee<sup>1,6,\*</sup>

**IL6 is associated with GWI Neuropathology in mouse models of GWI**

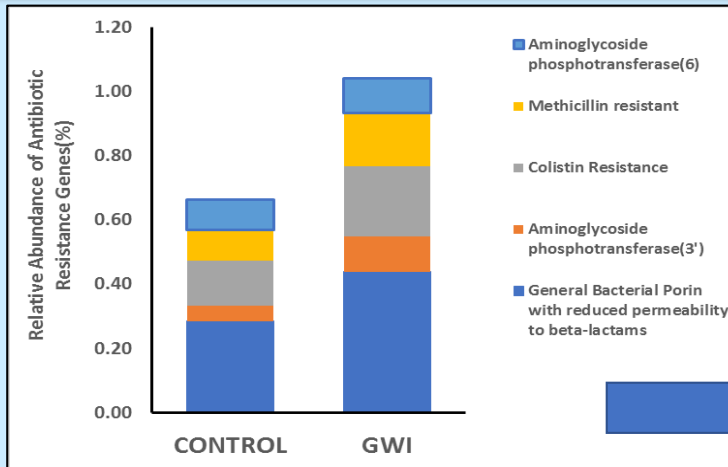


## The Gut Resistome

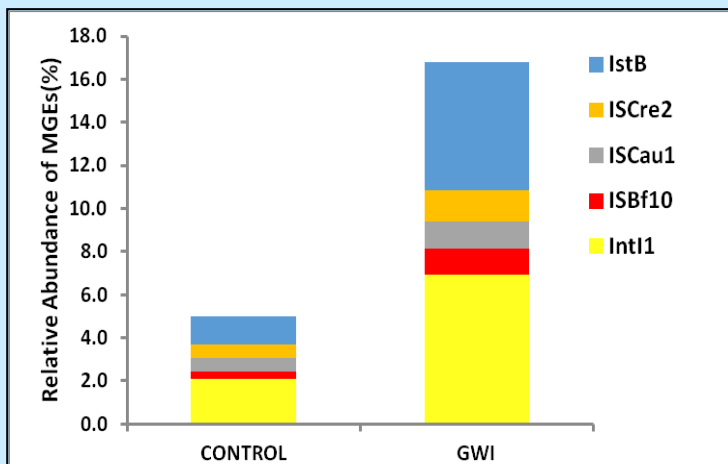
### Functions and Implications in GWI

#### Challenges

- **Prolonged Antibiotic use**
- **Chronic Gastrointestinal disturbances**
- **Obesity**
- **Infectious Disease (like COVID 19, nosocomial infection)**



Difference in ARGs



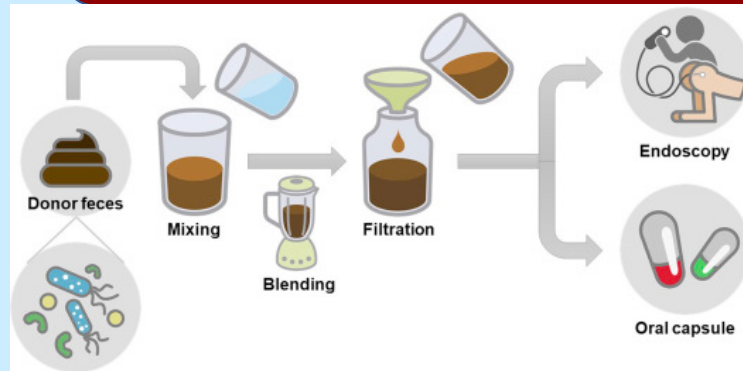
Difference in MGEs

#### ARG

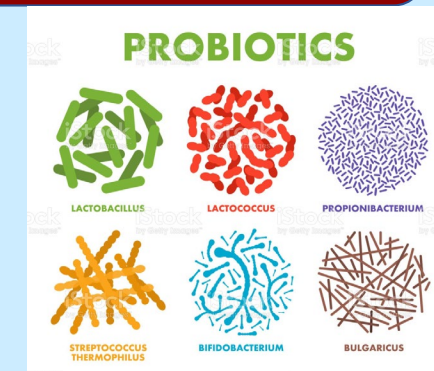
- **Aminoglycoside phosphotransferase (APH 6)** – inactivates aminoglycoside antibiotics (Kanamycin A, Gentamycin). Prolonged use among GWI veterans may lead to increased resistance to aminoglycoside antibiotics like Gentamycin.
- **Methicillin Resistant-** Produces altered penicillin (PBP2) binding protein, decreases the affinity towards  $\beta$ -lactam antibiotics. Altered PBP2 worsens GWI pathology by inactivating  $\beta$ -lactam antibiotics.
- **Colistin Resistance** - Modifies the bacterial outer membrane, Use of colistin antibiotic for treatment of combat wound and infection in war veterans increases resistance in this reservoir of patients for potential nosocomial transmission.
- **General bacterial porin** with reduced permeability to beta lactams - Decreased susceptibility to  $\beta$ -lactams render limitations of the use of  $\beta$ -lactam antibiotics like Carbapenem, Cephalosporin to combat veterans or elderly veterans.

1. **Susceptibility to acquired infection: Sepsis, Cytokine storm.**
2. **Susceptibility to altered immune complication: COVID 19, Nosocomial infections.**
3. **Susceptibility to altered metabolic complications: IBD, NAFLD.**

## Possible Therapeutic Targeting of the Altered Resistome



### Fecal Microbiota Transplant



1. **Fecal Microbiota Transfer**
2. **Treatment with Probiotics e.g. *Bifidobacterium longum***
3. **Restrict antibiotic use identified in the resistome**



## Conclusions: The therapeutics scenario in the next 10 years



- 1. Chatterjee Lab has identified bacteriome and virome characteristics in both rodent models and Veterans of GWI**
- 2. Chatterjee Lab has extensively studied the role of pattern recognition receptors (TLR4s and HMGB1) in Gut Brain Axis**
- 3. Chatterjee Lab studies showed a distinct role of TNFR1 and IL6 in modulating Gut Brain Axis of GWI (Infliximab and Tocilizumab can be good candidates)**
- 4. Currently we have identified two plant derived compounds that target the TLR4 and TLR7 (Sparstolonin B, Andrographolide)**
- 5. We have identified another potential drug target (Resistome)**
- 6. Resistome is particularly important since GWI veterans are aging and the characterization of Resistome can answer their susceptibilities to future diseases.**



# ACKNOWLEDGEMENTS



## Chatterjee Lab Post-docs & Ph.D. Students



Dr. Ratanesh Seth



Dr. Ayan Mondal



Dr. Somdatta Chatterjee



Dipro Bose



Punnag Saha



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- Dr. Efrem Lim, ASU.
- Dr. Jie Li, UofSC.
- Dr. Kimberly Sullivan, Patricia Janulewicz, (BBRAIN) Dept. of Environmental Health, Boston University School of Public Health.

