The **S**urveillance **H**ighlighting **E**xisting/Emerging **R**esistance **L**imiting **O**ur ***C***. *difficile* **K**nowledge (SHERLOCK) Project

Background:

Clostridioides difficile infection (CDI) is the most common healthcare-associated infection in the United States (U.S.) causing nearly a half-million cases and at least 12,800 deaths annually.[1, 2] Given the significant burden on the U.S. healthcare system, *C. difficile* has been classified as an urgent threat by the U.S. Centers for Disease Control and Prevention (CDC).[2, 3] CDI is unique in that diagnosis is made via the identification of toxins in stool rather than by bacterial growth. Therefore, routine antibiotic susceptibility testing is not performed on *C. difficile* isolates. Instead, active surveillance programs are required to identify new epidemic and/or antibiotic-resistant strains. While a U.S.-based *C. difficile* surveillance program has been established by the CDC, it has many limitations.[4] Their program consists of only 35 counties in ten states and is not representative of the entire U.S. Furthermore, the CDC does not perform antibiotic susceptibility testing or whole genome sequencing (WGS), which means they are unable to detect emerging antibiotic resistance and elucidate corresponding resistance mechanisms. Lastly, the CDC does not perform a robust clinical outcomes assessment to determine the effect of *C. difficile* strain, antibiotic resistance, and treatment on patient outcomes. A comprehensive, multistate surveillance program in the U.S. that combines phenotypic/genotypic resistance analyses and a robust clinical outcomes assessment is therefore desperately needed.

Study overview:

Our research group, led by Dr. Kevin W. Garey, has been funded by the U.S. CDC and the National Institutes of Health (NIH) to perform active surveillance of *C. difficile* in the state of Texas.[5-8] Dr. Garey has an ongoing collaboration with two large health systems in the Houston, Texas area which allows us to prospectively identify and collect leftover stool samples from patients diagnosed with CDI for high-level translational research studies in a centralized research laboratory at the University of Houston. These studies include *C. difficile* growth, strain typing, antibiotic susceptibility testing, WGS, and quantitative reverse transcription polymerase chain reaction (qRT–PCR).[5-11] In addition, we collect corresponding clinical data, which allows us to investigate the effects of *C. difficile* strain, antibiotic resistance, and treatment on patient outcomes.[8] We intend to expand this established surveillance program to multiple states.

Our long-term goal is to create a comprehensive, multistate surveillance program that improves upon the CDC's program. This surveillance program will allow us to identify *C. difficile* outbreaks while at the same time studying the effect of antibiotic resistance on patient outcomes. To achieve this goal, we are offering a strain typing service for *C. difficile* isolates using our validated fluorescent PCR ribotyping protocol.[5, 12] All isolates collected for this service will also undergo antibiotic susceptibility testing, WGS, and qRT–PCR to detect emerging antibiotic resistance and elucidate corresponding resistance mechanisms. Participating institutions will get a customized strain typing report of their local isolates on an annual basis. This report will provide detailed information about their local *C. difficile* epidemiology, including information on emerging *C. difficile* strains and antibiotic susceptibility. You can think of this report as a local *C. difficile* antibiogram.

Methods:

General overview

All participating institutions will be asked to collect and ship a minimum of 40 leftover, *C. difficile*-positive stool samples from nonduplicate patients each calendar year. The collection of stools for *C. difficile* testing will be conducted as part of routine clinical care per individual hospital algorithms, and all samples will be de-identified

prior to shipping to the University of Houston for laboratory experiments. In addition, each site will be asked to collect corresponding clinical data for each sample. Each individual site will maintain an encrypted key on an institution computer linking each stool sample to the patient's identify and clinical data.

Site responsibilities

Identification and collection of stool samples

Each participating institution will be asked to identify and collect a minimum of 40 stool samples from nonduplicate adult patients (age ≥18 years) that test positive for *C. difficile* on an annual basis. The collection of leftover stools should be done only after all clinical tests have been completed. Fortunately, there is no minimum volume requirement for samples as the spores of *C. difficile* are present in minute amounts of stool. All samples must be de-identified prior to shipping to the University of Houston for laboratory experiments. The only marking left on each container should be a site-specific code used to track each sample. This code will be provided to each site and will be used to match each stool sample to its corresponding clinical data. Each individual site will maintain the encrypted key on an institution computer.

Storage and shipping

A standardized protocol for shipping samples is provided in **Appendix A**. As the rate at which samples accumulate will be highly variable between sites, shipments will be sent at the site investigators' convenience. Fortunately, the spores of *C. difficile* are hardy, so there is no rush to ship samples. All samples should remain refrigerated prior to shipping; thus, the shipping schedule may be based on the storage capabilities of the site. All shipping supplies will be provided, and shipping costs covered for sites participating in this study.

Data collection

As previously mentioned, each site will maintain an encrypted key on an institution computer linking each stool sample to the patient's clinical data using a site-specific code. Each local investigator will use their encrypted key to determine which electronic medical records (EMRs) must be accessed. The EMRs will be reviewed for demographic information, laboratory data, medication administration records, and clinical outcomes. Study data will be collected and managed using a secure, web-based software platform designed to support data capture for research studies (e.g. REDCap (Research Electronic Data Capture)). No individually identifiable health information will be collected. A list of variables to be collected is provided in **Appendix B**.

Regulatory requirements

All samples and data will be de-identified. No individually identifiable health information will be collected as part of this project. This project has undergone institutional review board (IRB) review at High Point University, and a copy of the approval letter will be sent to each participating institution.

University of Houston responsibilities

C. difficile isolation: C. difficile will be isolated from positive stool samples. Stool samples will then be discarded.

Strain typing: Fluorescent polymerase chain reaction (PCR) ribotyping will be used to differentiate strains of *C. difficile*.

Antibiotic susceptibility testing: Antibiotic susceptibility of *C. difficile* to metronidazole, vancomycin, and fidaxomicin will be assessed using the broth microdilution method.

Whole genome sequencing (WGS): Whole genome sequencing will be performed to screen *C. difficile* isolates for the presence of virulence genes, including antibiotic resistance genes.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR): Quantitative reverse transcription polymerase chain reaction will be performed to determine which *C. difficile* isolates are actively expressing virulence genes identified via WGS.

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