Step I: White Paper Application

Application Guidelines

1. The application should be submitted electronically per requirements via the web site of any of the NIAID Genomic Sequencing Centers for Infectious Diseases. Include all attachments, if any, to the application.
2. There are no submission deadlines; white papers can be submitted at anytime.
3. GSC personnel at any of the three Centers can assist / guide you in preparing the white paper.
4. Investigators can expect to receive a response within 4-6 weeks after submission.
5. Upon approval of the white paper, the NIAID Project Officer will assign the project to a NIAID GSC to develop a management plan in conjunction with the participating scientists.
White Paper Application

**Project Title:** *Escherichia coli* as human pathogen: Uncomplicated urinary tract infection (UTI) and UTI induced bacteremia

**Authors:**

**Primary Investigator Contact:**

<table>
<thead>
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<th>Niels Frimodt-Moller</th>
</tr>
</thead>
<tbody>
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1. **Executive Summary (Please limit to 500 words.)**

Provide an executive summary of the proposal.

*Escherichia coli* is a major human pathogen, causing up to 80% of uncomplicated UTI and 30% of bacteremia cases. *Escherichia coli* is the most prominent cause of both community-acquired (CA) and nosocomial (NA) bloodstream infections worldwide, and the associated rate of mortality due to sepsis is high (Laupland *et al.*, 2008). In the U.S., *E. coli* related urinary tract infections kill ~7200 persons annually, and the annual mortality associated with *E. coli* bacteremia in the U.S. is around 36,000-40,000 (Russo & Johnson, 2003).

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in clinical practice and account for significant morbidity and high medical costs (Foxman, 2002). During any single year, 11% of women aged 18 and older develop UTI, and the lifetime risk of UTI among women is 60% (Foxman *et al.*, 2000). *Escherichia coli* is the most predominant pathogen causing 80–90% of community-acquired UTIs and more than 30% of nosocomially acquired UTIs (Ferry *et al.*, 2004; Kahlmeter, 2000; Bouza *et al.*, 2001). Recurrent UTIs (RUTIs) are reported in 16–25% of women within 6 months of an UTI episode and in 40% of women within one year of an UTI episode, and thus pose a major problem (Foxman, 1990; Foxman *et al.*, 1995; Ikaheimo *et al.*, 1996; Karkkainen *et al.*, 2000). Although most common among women, UTI is also a significant problem among men, who can experience cystitis, pyelonephritis, acute and chronic prostatitis and febrile UTI (Lipsky *et al.*, 1989; Ulleryd, 2003).

We propose sequencing several collections of *E. coli* urinary and blood isolates in order to provide insight into:

1) the genetic determinants associated with infection of the urinary tract both among women and men.
2) the genetic determinants associated with the relapse and persistence of *E. coli* in spite of treatment for UTI.
3) the genetic determinants associated with the dissemination to the blood.
Such knowledge could be used to predict the likelihood that patients with uncomplicated UTI will be at risk for persistence/relapse or spread to bloodstream as well as identify risk factors in the fecal E. coli of healthy people. The data produced will increase our understanding of the mechanisms of E. coli pathogenesis. Further, this data may be used to develop intervention strategies to prevent and treat infections with this pathogen.

2. Justification

Provide a succinct justification for the sequencing or genotyping study by describing the significance of the problem and providing other relevant background information.

This section is a key evaluation criterion.

1. State the relevance to infectious disease for the organism(s) to be studied; for example the public health significance, model system etc.

2. Are there genome data for organisms in the same phylum / class / family / genus? What is the status of other sequencing / genotyping projects on the same organism including current and past projects of the NIAID GSC? Provide information on other characteristics (genome size, GC content, repetitive DNA, pre-existing arrays etc.) relevant to the proposed study. Have analyses been performed on the raw data already generated/published? If additional strains are proposed for a species, please provide a justification for additional strains?

3. If analyses have been conducted, briefly describe utility of the new sequencing or genotyping information with an explanation of how the proposed study to generate additional data will advance diagnostics, therapeutics, epidemiology, vaccines, or basic knowledge such as species diversity, evolution, virulence, etc. of the proposed organism to be studied.

Recurrent UTI:

Treatment, host and pathogen characteristics are considered to have an impact on development and frequency of RUTI; however, many aspects are unclear or poorly described. Up to 77% of RUTIs are caused by E. coli identical to the primary infecting E. coli; however, characteristics of E. coli associated with relapse of UTI remain poorly defined (Ejrnaes et al., 2006; Russo et al., 1995; Skjøt-Rasmussen et al., 2011). Phylogenetic group B2 has been found to be associated with strains causing persistence or relapse of UTI (Johnson et al., 2007). A wide variety of virulence factors (VFs) have been associated epidemiologically or experimentally (in vivo) with uropathogenic E. coli (UPEC) including adhesins, iron uptake systems, toxins and protectins; however, only a few minor, older studies have addressed the relation between VFs and relapse of UTI in women (Johnson & Russo, 2005; Foxman et al., 1995; Johnson et al., 2001).

Although the fecal and vaginal flora were thought to constitute the reservoir for RUTI-causing E. coli strains, this view has become increasingly challenged by the finding that E. coli can invade and replicate within the murine bladder forming biofilm-like intracellular bacterial communities (IBCs) (Anderson et al., 2003; Garofalo et al., 2007). These IBCs
dissociate and E. coli flux out and ultimately establish quiescent intracellular reservoirs that may represent stable reservoirs for RUTI (Mysorekar et al., 2006; Schilling et al., 2002). The IBC pathogenic cycle has not been studied in humans; however, recently exfoliated IBCs were detected in urine from women with acute uncomplicated cystitis which supports the presence of the IBC pathway and occurrence of an intracellular bacterial niche in some women with UTI (Rosen et al., 2007).

IBC formations contain biofilm-like structures and it has been shown that E. coli causing persistence or relapse exhibited significantly increased biofilm formation on plastic surface in vivo, but the prevalence of virulence factors associated with biofilm formation (e.g. agn43) has not been examined in relation to E. coli causing relapse (Ejrnaes, K., A. Reisner, B. Lundgren, S. Ferry, T. Monsen, S. Holm, E. Zechner, and N. Frimodt-Moller, submitted for publication; Soto et al., 2006). Recently, heme- and siderophore-associated iron have been shown to play a key role in IBC development in mice; however, it is not known whether this observation is reflected in an association between prevalence of genes coding for different iron uptake systems and E. coli strains causing relapse of UTI (Reigstad et al., 2007).

**Male UTI:**
Many aspects on male UTI are unclear or poorly described. Knowledge of male UTI is less than that of female UTI even though it is estimated that one-third of all 80-year-old men will have had an episode of bacturia (Lipsky, 1989). It is generally believed, that all UTIs in men must be considered complicated since the infections results from an anatomic or functional anomaly, however there is little evidence to support this hypothesis. A better understanding of the pathogenesis of UTI in men is needed in order to identify possible targets for preventive and protective measures.

**Bacteremia:**
Around 17-37% of invasive bacteremic E. coli infections are due to Extraintestinal Pathogenic E. coli (ExPEC), which often originate from an infection in the patients’ urinary tract (Olesen et al., 1995; Russo & Johnson, 2003). ExPEC possess a wide variety of specialized virulence factors (VFs), however the requirements for bacterial invasion of the bloodstream are yet undetermined (Ron, 2006). Non-presence of virulence factors such as adhesins has been shown to relate to infections in patients with immune depression (Maslow et al., 1993). However, few studies have investigated the decisive virulence factors involved in UTI pathogenic E. coli strains invading the blood stream (Johnson & Stell, 2000; Moreno et al., 2005; Rijavec et al., 2008). In order to identify and develop new targets for antimicrobial agents or develop a vaccine, it is necessary to understand the pathogenesis and virulence of bacteremic E. coli.

**Previous work:**
The Broad Institute has recently completed sequencing of over eighty five commensal E. coli. A major goal of that work was to provide a commensal, non-pathogenic context in order to better understand the genomics of E. coli pathogenesis. Specifically, this project sequenced nine commensal strains from the ST95 clone, which is one of the most predominant UTI clones. Analysis of the proposed UTI and bacteremia isolates, in conjunction with those commensal isolates, will enable us to identify pathogenesis-specific loci. Other GSCIDs have focused on other pathogenic groups of E. coli, such as the
O157:H7 serotype. A comparison with those data will further refine our understanding of how genetic variation contributes to specific *E. coli*-related disease syndromes.

The study has major relevance for the understanding of the virulence potential of *E. coli* isolated from healthy persons or from patients with uncomplicated UTI. These data ultimately could aid the prediction of the course of infection or complications, or lead to intervention strategies in *E. coli*-related disease. To our knowledge, similar studies using a well-designed *E. coli* collection from UTI and bacteremia have not been performed before, especially at this scale. The public health benefit from such a project concerning this particular pathogen has enormous potential.

### 3. Rationale for Strain Selection

4. Provide the rationale behind the selection of strains and the number of strains proposed in the study. The focus of the program is on potential agents of bioterrorism or organisms responsible for emerging or re-emerging infectious diseases. Non-select agents or non-pathogenic organisms will be considered when they can provide insight into these scientific areas.

*E. coli* strains were isolated from three populations:

1. **Umeå collection:** Female UTI patients with uncomplicated UTI experiencing either cure (*n* = 30), relapse (*n* = 46) or persistence of infection (*n* = 32)

2. **Køge collection:** Male UTI patients with uncomplicated (*n* = 7) or recurrent UTI (*n* = 3) and female patients experiencing recurrent UTI (*n* = 10)

3. **Hvidovre collection:** Bacteremia patients (*n* = 197)

This will yield a total of 315 *E. coli* strains for sequencing.

**Female recurrent UTI (Umeå collection):**

The Umeå collection of female recurrent UTI *E. coli* strains were obtained through a study performed by Ferry et al., 2007, where about 1200 women with uncomplicated UTI were randomized to one of four groups: Three groups received mecillinam orally for 3 or 7 days, while the fourth group received placebo. Control visits occurred 1 week after treatment and 4 weeks after treatment.

The patients consisted of a cohort of women with uncomplicated urinary tract infection, who delivered three urine samples:

1) at the first visit at the family doctor (general practitioner), where the diagnosis of UTI was confirmed,

2) first control sample one week after the end of antibiotic treatment, and

3) second control sample one month after the end of treatment.

Based on PFGE typing of *E. coli* isolates, patients could be divided in three groups: i) cure (no growth in control samples); ii) relapse (no growth in the first control sample, but growth of the same PFGE type in the second of control samples); iii) persistence (growth in the first control sample of the same PFGE type). In all, 57 *E. coli* strains from cured patients and 88 *E. coli* strains from patients with persistence/relapse were isolated.

Table 1 shows the distribution of *E. coli* strains in the mecillinam treated groups, where cure, persistence or relapse was discerned by PFGE-typing (100% band similarity was used as a criterion for identity). Overall, around 80% similarity was found for strains causing relapse or persistence (Ejrnæs et al., 2006). This illustrates, that most strains remaining in the urine were...
the same as the originally infecting strain. It is therefore highly likely that these strains were surviving in a urinary tract reservoir, unreached by antibiotic treatment; experimental mouse UTI studies have shown that E. coli infecting the bladder are not removed by antibiotic treatment (Hvidberg et al., 2000). The reason for this can be biological biofilm formation or some other factor protecting the bacteria from antibiotic present both in urine and in blood.

Table 1.
E. coli distributed according to PFGE typing: “Same” means 100% similar in PFGE-profile.

<table>
<thead>
<tr>
<th>Culture Result</th>
<th>Course of Infection</th>
<th>Pivmecillinam Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 155</td>
</tr>
<tr>
<td>Initial Visit</td>
<td>1st follow-up</td>
<td></td>
</tr>
<tr>
<td>E. coli Negative</td>
<td>Negative</td>
<td>400 mg BID for 3 days</td>
</tr>
<tr>
<td>E. coli Negative</td>
<td>Missing</td>
<td>200 mg BID for 7 days</td>
</tr>
<tr>
<td>E. coli Same E. coli</td>
<td>Same E. coli</td>
<td>200 mg TID for 7 days</td>
</tr>
<tr>
<td>E. coli Same E. coli</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>E. coli Same E. coli</td>
<td>Missing</td>
<td></td>
</tr>
<tr>
<td>E. coli Negative</td>
<td>Same E. coli</td>
<td></td>
</tr>
<tr>
<td>E. coli Negative</td>
<td>New E. coli</td>
<td></td>
</tr>
<tr>
<td>E. coli New E. coli</td>
<td>New E. coli</td>
<td></td>
</tr>
<tr>
<td>E. coli New E. coli</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>E. coli New E. coli</td>
<td>Missing</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Course of Infection</th>
<th>1st follow-up</th>
<th>2nd follow-up</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure</td>
<td>37</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Persistence</td>
<td>15</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Persistence</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Persistence</td>
<td>12</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Relapse</td>
<td>46</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Reinfection</td>
<td>14</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Reinfection</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Reinfection</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Reinfection</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>46</td>
<td>47</td>
</tr>
</tbody>
</table>

aCourse of infection according to PFGE results by Ejrnaes et al. (5)
b8-10 days post inclusion
c35-49 days post inclusion
dBID, twice a day; TID, three times a day
eSame E. coli as the primary infecting E. coli at inclusion according to PFGE (5)
fE. Coli different from the primary infecting E. coli at inclusion according to PFGE (5)

**Male and female (recurrent)UTI (Køge collection):**
Between December 2005 and April 2006, consecutive urine culturing on patients with symptoms of UTI was performed at a primary care clinic (not associated to any hospital). E. coli isolates were collected from 102 patients with community-acquired UTI, and 13 of these patients (10 women and 3 men) experienced recurrent UTI (Skjøt-Rasmussen et al., 2011). Besides the 3 men experiencing RUTI, 7 male patients experienced uncomplicated UTI. The following isolates from this strain collection will be included in the study: Isolates from male uncomplicated (n = 7) and recurrent UTI (n = 3) and female recurrent UTI (n = 10).

**Bacteremia (Hvidovre collection):**
This strain collection consists of 197 E. coli isolates from the blood of 196 adult patients with
both bacteremia and bacturia. From one patient, two \textit{E. coli} blood isolates were cultured. Isolates were collected from January 2003 through May 2005 from all patients older than 18 years admitted to four hospitals in Copenhagen. Isolates represent all consecutive episodes of \textit{E. coli} bacteremia with bacturia, where a positive \textit{E. coli} urine culture was performed +/- three days within the blood culture date.

References:
- Karkkainen, U. M., R. Ikaheimo, M. L. Katila, and A. Siitonen. 2000. Recurrence of urinary tract infections...
4a. Approach to Data Production: Data Generation

5. State the data and resources planned to be generated. (e.g. draft genome sequences, finished sequence data, SNPs, DNA/protein arrays generation, clone generation etc.)

As we wish to identify novel genetic elements that are potentially unique to certain disease states, persistence, and spread, we will generate draft *de novo* genome sequences for all proposed strains using the Illumina platform. Our experience in sequencing numerous *E. coli* strains suggests that a major issue is that many genes of interest, such as those associated with virulence and antibiotic resistance, are associated with transposable elements and other repetitive motifs. To overcome this problem, we will use large jumping library insert sizes (~5 kb) that should enable us to scaffold over the repeated elements.
4b. Approach to Data Production: Data Analysis

6. Briefly describe the analysis (value-add) envisioned to be performed subsequently by the community and the potential to develop hypotheses driven proposals given the datasets and resources produced by this work.

**UTI (Umeå and Køge collections):**

**Umeå strains:** Sequencing and comparison of strains related to cure (n = 37) vs. strains related to persistence (n = 32) or relapse (n = 46) may reveal genetic factors – so far unknown – which would be related to cure or relapse. Total sequencing of the bacterial genomes may reveal genes, clusters of genes such as pathogenicity islands or others that would differentiate relapsing strains from those found in patients, who were cured by mecillinam treatment. All strains were susceptible to mecillinam, so resistance towards the study drug was not an issue.

**Køge strains:** Sequencing and comparison of strains isolated from male UTI (n = 7+3) and comparison with female strains (n = 10; and from Umeå, see above) may reveal genetic factors related to male UTI and recurrent UTI both among men and women.

**Table 2. Summary of E. coli UTI strains to be sequenced.**

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female patients cured</td>
<td>37</td>
</tr>
<tr>
<td>Female patients with persisting E. coli</td>
<td>32</td>
</tr>
<tr>
<td>Female patients with relapse</td>
<td>46</td>
</tr>
<tr>
<td>Female patients with RUTI</td>
<td>10</td>
</tr>
<tr>
<td>Male patients with RUTI</td>
<td>3</td>
</tr>
<tr>
<td>Male patients with uncomplicated UTI</td>
<td>7</td>
</tr>
</tbody>
</table>

**Outcome:**

The detection of genes/clusters that are predictable of relapse/persistence would enable physicians to stratify patients prior to treatment of UTI, so that appropriate treatment could be directed to patients deemed at higher risk for complications. Such treatments could include longer duration of antibiotic treatment or choice of antibiotics, which are particularly effective in curing E. coli infection residing in the bladder. This stratification could be based on rapid SNP detection or gene presence in the primary culture, which again would be suggested mandatory in patients with symptoms of UTI at the primary care level. Currently, many general practitioners do not perform urinary culture in primary care, but treat the patients empirically with a broad spectrum antibiotic and await whether the patient is cured or returns with symptoms, where a urine culture is then first performed. A genomically-informed algorithm such as that suggested could prevent the widespread use of broad-spectrum antibiotics, currently felt to be fueling the increasing antibiotic resistance problem, and tailor therapy to each patient.

Further, sequencing of the present strain collection would lead to detailed insight into the genome of E. coli causing UTI. As described in the Justification section, these data, when compared to the large commensal collection of E. coli currently sequenced by the Broad Institute, as well as other E. coli-related disease syndromes, will help us pinpoint the genetic underpinnings of both male and female UTI infection.

**Bacteremia (Hvidovre collection):**
The objectives of this study are to characterize *E. coli* causing bacteremia as a complication of UTI with respect to the presence of VFs and antimicrobial resistance. Sequencing of these strains would be a unique opportunity to study and explore possible differences in the prevalence and distribution of all present bacterial genomic content such as virulence factor genes, antimicrobial resistance genes etc. among *E. coli* causing UTI and bacteremia. Furthermore, the *E. coli* strains in this collection can be compared with other *E. coli* strains – strains from healthy humans and strains causing both uncomplicated and complicated UTI.

**Outcome:**
Description of genes or gene-clusters, which are peculiar for *E. coli* causing bacteremia, will have several important consequences for handling patients with UTI or bacteremia: i) Detection of such genes in *E. coli* isolated in urine from UTI will enable particular attention to this kind of infection, effective antibiotics to cope with the infection and prevention of bacteremia. ii) Knowledge of important virulence factors for invasive strains may lead to preventive measures such as vaccines; iii) The existence of particular *E. coli* strains causing bacteremia and originating from UTI may provide the clinicians with one or more tags, which can enable the detection of the focus of infection causing bacteremia, a prerequisite for effective treatment of bacteremia.

5. **Community Support and Collaborator Roles:**

7. Provide evidence of the relevant scientific community's size and depth of interest in the proposed sequencing or genotyping data for this organism or group of organisms. Please provide specific examples.

In light of the novel collections described here, this proposal should be of broad interest to ID specialists, UTI experts, and those interested in developing diagnostics for primary care.

8. List all project collaborators and their roles in the project.

**Line Skjøt-Rasmussen (Dept. for Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark).** Line Skjøt-Rasmussen is performing the PhD-project regarding virulence in Extraintestinal Pathogenic *Escherichia coli* based on the Hvidovre and Køge *E. coli* strain collections. She will perform analysis and interpretation of the *E. coli* genomes, along with the other analyses of this project.

**Niels Frimodt-Møller (Dept. for Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark).** Niels Frimodt-Møller is supervisor for Line Skjøt-Rasmussen on her PhD-project and is the major developer of this project. He will supervise Line during the analysis and assist with interpretation of results.

**Karen Ejrnæs (Dept. for Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark; Dept. of Clinical Microbiology, Hvidovre Hospital, Copenhagen, Denmark; Dept. of Pathology, Herlev Hospital, Copenhagen, Denmark).** Karen Ejrnæs has performed the PhD-project “Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*” based on the...
Umeå *E. coli* strain collection. Also, she collected the Hvidovre *E. coli* strain collection. She will assist with interpretation of results.

**Lotte Jakobsen (Dept. for Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark).** Lotte Jakobsen has completed the PhD-project “Evaluation of the possible association between *Escherichia coli* from animals and meat with *E. coli* causing urinary tract infections in humans” based on the Køge *E. coli* strain collection. She will assist with interpretation of results.

**Paal Skytt Andersen (Dept. for Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark).** Paal Skytt Andersen has experience in whole genome sequencing, annotation, alignment and will assist and supervise in the analysis of the genomes.

**Bettina Lundgren (Dept. of Clinical Microbiology, Hvidovre Hospital, Copenhagen, Denmark; The Centre of Diagnostic Investigations, Rigshospitalet, Copenhagen, Denmark).** Bettina Lundgren is supervisor for Line Skjøt-Rasmussen on her PhD-project. She will supervise Line during the analysis and assist with interpretation of results.

**Tor Monsen and Sven Ferry (Department of Clinical Microbiology, Umeå, Sweden).** Tor Monsen and Sven Ferry collected the Umeå strain collection of female recurrent UTI *E. coli* strains. They will assist with interpretation of results.

**Michael Feldgarden (The Broad Institute).** Dr. Feldgarden has organized several large-scale, GSC-funded population genomics projects, including one focused on ~100 *E. coli* commensal genomes. He is also involved with the analysis of the data from these projects.

9. **List availability of other funding sources for the project.**

Funding of the project has been provided from the Danish Medical Research Council (grant number 22-02-0373 ct/mp), the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), and the University Hospital in Hvidovre, Copenhagen, Denmark.

6. **Availability & Information of Strains:**
10. Indicate availability of relevant laboratory strains and clinical isolates. Are the strains/isolates of interest retrospectively collected, prepared and ready to ship?

Note: If samples are prospectively prepared the GSC can provide protocols and recommendation based on the Centers past experiences. The samples must however meet minimum quality standards as established by the Center for the optimal technology platform (sequencing/ genotyping) to be used in the study.

All strains are readily available and DNAs can be prepared.

11. Attach relevant information, if available in an excel spreadsheet for multiple samples: e.g.

- Name
- Identifier
- Material type (DNA/RNA/Strain)
- Genus
- Species
- Specimen / Strain
- Isolation source
- Isolated from
- Select agent status
- International permit requirement
- BEIR/ATCC repository accession number
- Other public repository location
- Other public repository identifier
- Sample provider’s name
- Sample provider’s contact

12. What supporting metadata and clinical data have been collected or are planned on being collected that could be made available for community use?

Available metadata are data concerning the patients’ gender and age, source of the *E. coli* isolate (urine or blood) and year of collection.
7. Compliance Requirements:
7a. Review NIAID’s Reagent, Data & Software Release Policy:
NIAID supports rapid data and reagent release to the scientific community for all sequencing and genotyping projects funded by NIAID GSC. It is expected that projects will adhere to the data and reagent release policy described in the following web sites.
http://www3.niaid.nih.gov/research/resources/mscs/data.htm
<Each Center to include their website that describes/points to the guidelines>
Once a white paper project is approved, NIAID GSC will develop with the collaborators a detailed data and reagent release plan to be reviewed and approved by NIAID.

Accept ☐ Decline ☐

7b. Public Access to Reagents, Data, Software and Other Materials:

13. State plans for deposit of starting materials as well as resulting reagents, resources, and datasets in NIAID approved repositories. Sequencing projects will not begin until the strain is deposited into NIAID funded BEI repository (http://www.beiresources.org/). This includes web based forms are completed by the collaborator and received by the NIAID BEI (http://www.beiresources.org/).

All strains current unavailable through public repositories will be deposited at BEI. All data produced will be published and available to the public.

7c. Research Compliance Requirements
Upon project approval, NIAID review of relevant IRB/IACUC documentation is required prior to commencement of work. Please contact the GSC Principal Investigator(s) to ensure necessary documentation are filed for / made available for timely start of the project.

Investigator Signature:

Investigator Name: Date:
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