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Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance
The SCOUT Study: "Short Course Therapy for Urinary Tract Infections in Children"

IND Sponsor: DMID

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STATEMENT OF COMPLIANCE

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia and Children's Hospital of Pittsburgh's Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this IRB-approved protocol, will obtain consent and assent, and will report adverse events (AEs) in accordance with Children's Hospital of Philadelphia and Children's Hospital of Pittsburgh's IRB Policies and Procedures and all federal requirements. The Principal Investigator will assure that no changes to the protocol will take place without prior agreement from the sponsor and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the study participants. The Principal Investigator will promptly report to the IRB and the sponsor any changes in research activity and all unanticipated problems involving risk to human subjects, or others. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

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LIST OF ABBREVIATIONS

°C	Degrees centigrade
AAP	American Academy of Pediatrics
AE	Adverse Event/Adverse Experience
APN	Acute pyelonephritis
BDMC	Biostatistics and Data Management Core
CHOP	Children's Hospital of Philadelphia
CDM	Clinical Data Manager
CFR	Code of Federal Regulations
CFU/mL	Colony Forming Units per milliliter
CLSI	Clinical Lab and Standards Institute
CONSORT	Consolidated Standards of Reporting Trials
CRA	Clinical Research Assistant
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Event
CTC	Clinical Trial Center
CTRC	Clinical and Translational Research Center
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
DMP	Data Management Plan
DMSA	Dimercaptosuccinic acid
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
ED	Emergency Department
EHR	Electronic Health Record
ESBL	Extended Spectrum Beta Lactamases
FDA	Food and Drug Administration
FWA	Federal wide Assurance
F/SUTI	Febrile or Symptomatic Urinary Tract Infection
FUTI	Febrile Urinary Tract Infection
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive Care Unit

IDE	Investigational Device Exemption
IDS	Investigational Drug Service
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IVR	Interactive Voice Response System
ITT	Intention to Treat
JAMA	Journal of the American Medical Association
MedDRA ®	Medical Dictionary for Regulatory Activities
MOA	Memorandum or Memoranda of Agreement
MOP	Manual of Procedures
MOU	Memorandum or Memoranda of Understanding
N	Number (typically refers to subjects)
N/A	Not Applicable
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OC	Oracle Clinical
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PeRC	CHOP Pediatric Research Consortium
PHI	Protected Health Information
PI	Principal Investigator
PITT	Children's Hospital of Pittsburgh
PITTnet	PITT Practice Based Research Network
PK	Pharmacokinetics
PKU	Phenylketonuria
PMO	Program Management Office
QA	Quality Assurance
QC	Quality Control
RDC	Remote Data Capture
RIS	Research Information Systems
RNA	Ribonucleic acid
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical Analysis Plan
SCOUT	Short COurse Therapy for Urinary Tract Infections in Children
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure

SP	Special Publication
SUTI	Symptomatic Non-febrile Urinary Tract Infection
TOC	Test of Cure
TBD	To Be Determined/Developed
TCP/IP	Transport Control Protocol / Internet Protocol
TMP-SMX	Trimethoprim-Sulfamethoxazole
UA	Urinalysis
UPENN	University of Pennsylvania
UPS	United Parcel Service of America
UTI	Urinary Tract Infection
VUR	Vesicoureteral reflux
WARP	Westat Automated Report Portal
WBC/hpf	White Blood Cells per High Powered Field
WBC/mm^3	White Blood Cell per cubic millimeter

PROTOCOL SUMMARY

Title:

Targeted Clinical Trials to Reduce the Risk of Antimicrobial Resistance: The SCOUT Study: "Short Course Therapy for Urinary Tract Infections in Children." The SCOUT Study is a multi-center, centrally randomized, double-blind, placebo-controlled non-inferiority clinical trial of children ages two months (at least 36 weeks gestation from birth for subjects < two years of age) to 10 years with a confirmed diagnosis of a urinary tract infection (UTI).

Phase:

Phase II

Population:

Children: ages two months (at least 36 weeks gestational age from birth for subjects < two years of age) to 10 years with a confirmed urinary tract infection (UTI) diagnosis treated by one of four antibiotics (trimethoprim-sulfamethoxazole (TMP-SMX) or cefixime or cefdinir or cephalexin). The SCOUT Study will only enroll those children with documented clinical improvement (afebrile and asymptomatic) after initiation of antibiotic treatment. Subjects can be enrolled at any time after they initiate their originally prescribed antibiotic treatment, providing they meet all inclusion/exclusion criteria. However, all subjects will remain on their originally prescribed antibiotic treatment through the end of Day 5. Subjects will initiate their SCOUT study product beginning on Day 6. Subjects may be enrolled on Day 6 providing they have not started the Day 6 of their originally prescribed antibiotic treatment. Enrolled subjects that develop a fever or have worsening symptoms prior to taking the first dose of SCOUT therapy on Day 6 will be considered entry failures and withdrawn from the study.

A urinary tract infection (UTI) will be defined as the presence (in the medical record or by parent report) of:

(1) At least one of the symptoms consistent with the diagnosis of UTI including:

• Symptoms for all children (ages two months to 10 years):
- fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
- dysuria

• Additional symptoms for children > 2 years of age:
- suprapubic, abdominal, or flank pain or tenderness OR
- urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)

• Additional symptoms for children ≥ 2 months to 2 years of age:
- poor feeding OR

- vomiting

AND

(2) Pyuria based on urinalysis;
- $\geq$10 WBC/mm^3 (uncentrifuged specimen) OR
- $\geq$5 WBC/hpf (centrifuged specimen), OR
- Leukocyte esterase $\geq$ trace on dipstick.

AND

(3) Culture-proven infection with a single uropathogen:
- $\geq 5 \times 10^4$ CFU/mL (catheterized or suprapubic aspiration urine specimen) OR
- $\geq 10^5$ CFU/mL (clean void specimen)

Number of Sites:

There are two participating clinical trial centers (CTCs): Children's Hospital of Philadelphia (CHOP) and Children's Hospital of Pittsburgh (PITT). Children will be recruited in the primary and urgent care offices, emergency departments, and hospital wards from both CTCs.

Study Design:

The SCOUT Study is a multi-center, centrally randomized, double-blind, placebo-controlled non-inferiority clinical trial. The study will enroll about 746 children ages two months (at least 36 weeks gestational age for subjects < two years of age) to 10 years to evaluate 672 for the study's primary outcome measure. Enrolled subjects will be stratified based on presence of fever (a temperature of at least 100.4°F or 38°C measured anywhere on the body) at the initial presentation of UTI symptoms and by specific antibiotic therapy prescribed by the original treating clinician. At time of enrollment, children will be blindly randomized to the standard course of antimicrobial therapy arm (continue on antibiotics from Day 6-Day 10) or the short course antimicrobial therapy arm (receive placebo from Day 6-Day 10). Parents of subjects enrolled prior to completion of Day 5 of the original prescribed antibiotic prescription will be instructed to complete the prescribed treatment through the end of Day 5, and initiate SCOUT study therapy beginning on Day 6. Parents of subjects enrolled prior to Day 5 will be contacted by SCOUT study staff on Day 5 or Day 6 (prior to taking first dose of SCOUT study treatment on Day 6) to verify that the subjects do not have a fever or worsening of symptoms.

Study Duration:

Subjects will be enrolled over approximately a four and a half year period.

Subject Participation Duration:

The study period will begin on Day 6 after initial antibiotic therapy (Day 1 of SCOUT-directed treatment) is started and extend until the final follow-up phone call (four weeks after completing the study product therapy). Study duration for each individual subject will be approximately five weeks.

Description of Agent or Intervention:

The SCOUT Study will enroll children whose prescribing clinician initiated therapy with one of four antibiotics (trimethoprim-sulfamethoxazole sulfamethoxazole (TMP-SMX) or cefixime or cefdinir or cephalexin) for UTI in children across the two participating clinical sites. On occasion cefixime may not be available to the prescribing primary care physician. Under such circumstances it is likely that cefdinir will be prescribed as an alternative agent to cefixime instead. The two antibiotics have therapeutic equivalence. If a child initially prescribed cefdinir is enrolled, they will be randomized to either five additional days of cefixime or the cefixime placebo.

Children will take the medication prescribed by their physician for five days. Prior to taking the sixth day of medication, children will be enrolled in the SCOUT study and randomized to either the standard course arm or short course arm. Children randomized to the standard course arm will receive five additional days of the initially prescribed antibiotic. Children randomized to the short-course therapy arm will receive five additional days of a liquid placebo in an equivalent weight-based volume (same dosage mg/kg) to that of the antibiotic they would have received had they been in the treatment group for five additional days. The placebo will appear identical in color, taste, thickness, and consistency to the originally prescribed antibiotic. The study product kits will be similarly labeled with a numerical code that masks site investigators, site staff, parent(s)/guardian(s) and children to the formulation.

Objectives:

Primary Objective:

To determine if halting antimicrobial therapy in subjects who have exhibited clinical improvement 5 days after starting antibiotic therapy (short course therapy) have the same failure rate (symptomatic UTI) through TOC (visit Day 11-14) as subjects who continue to take antibiotics for an additional 5 days (standard course therapy).

Secondary Objectives:

To determine if short-course therapy compared to standard course therapy results in similar numbers of children experiencing a recurrent urinary tract infection (relapse and reinfection).

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with asymptomatic bacteriuria.

To determine if short-course therapy compared to standard course

therapy results in similar numbers of children with gastrointestinal colonization of antimicrobial resistant *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumoniae*).

To determine if short-course therapy compared to standard course therapy results in similar numbers of subjects presenting with clinical symptoms that may be related to UTI.

To determine if the number of subjects with positive urine culture prior to or at TOC is similar after short-course therapy compared to standard course therapy.

Description of Study Design:

Investigators at or affiliated with one of the two clinical centers will refer children treated for a UTI. Children will be recruited from three different sources: primary or urgent care sites, general pediatrics inpatient units and the emergency departments. Children who meet the eligibility criteria and whose parent(s)/guardian(s) agree to participate in the study will be scheduled for an enrollment visit. At the time of enrollment, children will be randomized to the standard-course arm or the short-course arm at a 1:1 ratio. Subjects may be enrolled at any time following initiation of antibiotic therapy, providing they meet all inclusion/exclusion criteria. However, all subjects will continue on the originally prescribed antibiotic treatment through the end of Day 5. Subjects enrolled Day 6 cannot have started the Day 6 of their originally prescribed antibiotic treatment. Any volunteer that is enrolled and subsequently develops a fever or has worsening symptoms prior to starting SCOUT therapy on Day 6 will be withdrawn from the study and considered an entry failure. For subjects enrolled prior to Day 5, confirmation from the parents will be obtained on Day 5 or Day 6 (prior to taking the first dose of SCOUT therapy on Day 6) that the subjects remain afebrile and have not had worsening of symptoms. Any volunteer that develops a fever or worsening symptoms prior to initiating study treatment will be considered an entry failure.

After enrollment, subjects will be scheduled for two follow-up visits and will receive a follow-up phone call at the end of the study:

Day 11 - 14 Test of Cure (TOC) Visit (*One – four days after completing the study product).* Subjects will be evaluated for clinical symptoms of UTI and a urine sample will be collected for analysis from all subjects. In addition, a stool sample will be obtained for assessment of antimicrobial resistant *Escherichia coli* (*E .coli*) and *Klebsiella pneumoniae (K. pneumonia).*

Day 24 - 30 *Outcome Assessment Visit (14 - 20 days after completing the study product).* Subjects will be evaluated for clinical symptoms of UTI. A urine sample will be collected only for those asymptomatic subjects for whom a urine sample could not be obtained at the TOC Visit and subjects that present with UTI symptoms at this visit. In addition, a stool sample will be obtained for assessment of antimicrobial resistant *Escherichia coli* (*E .coli*) and *Klebsiella pneumoniae (K. pneumonia).*

Day 38 - 44 Follow-Up Phone Call (*28-34 days after completing the study product*). Subjects will be contacted by phone and asked about the presence of UTI symptoms.

Follow-up for subjects that develop UTI throughout study

Throughout the study, if a subject develops UTI symptoms, he/she will be evaluated by a SCOUT physician or if preferred, the child's primary care provider. This Interim Visit will be performed only to determine whether the child has a UTI, so no stool specimen will be collected. If the child has pyuria and the urine culture is positive, the symptomatic child will stop the study product (if during the Day 6-10 period), and will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician. A SCOUT PI will follow up with the physician on the child's status.

Subjects that are diagnosed with a UTI while on study treatment and prior to the TOC Visit (between Day 6 and Day 11-14 TOC Visit) will be considered treatment failures and do not have to return for the TOC Visit but they will be asked to return for the Day 24-30 Outcome Assessment Visit and receive the Day 38-44 phone call. Similarly, subjects that are diagnosed with a UTI at any time after the TOC Visit through the Day 38-44 call will continue to return for the Outcome Assessment Visit and receive the Day 38-44 follow-up phone call. A stool specimen will still be collected at the Outcome Assessment Visit for these subjects diagnosed with UTIs (either before or after TOC).

Sub-studies

Seattle Children's Hospital R21

An R21-funded sub-study titled, "Differences in infecting and colonizing Enterobacteriaceae from short-course vs. standard therapy of pediatric UTI" is being performed with collaborators at Seattle Children's Hospital. This sub-study hypothesizes that both the antibiotic agent and the length of therapy will affect the patients' indigenous flora and thus affect both the likelihood of recovering E. coli and/or K.

pneumoniae from stool and the resistance phenotypes of the recovered isolates. The sub-study will utilize PCR- and sequence-based methods to characterize phylogenetic and resistance properties of the recovered isolates.

Carbapenem resistant enterobacteriaceae
For each enrolled research subject, the stool or perirectal swab collected at the Test of Cure visit will also be utilized to perform additional screening for the presence of carbapenem resistant enterobacteriaceae. The results of this additional screening will be used to determine the prevalence of colonization with carbapenem-resistant Enterobacteriaceae (overall and for specific pathogens) in ambulatory children after completion of treatment for UTI.

Microbiome pilot study
For each subject at CHOP who agrees to participate in the Microbiome pilot study, an additional perirectal swab and anal swab will be collected. The additional swabs will be collected at each visit and will be used to determine the composition of the subjects' microbiome. For subjects in diapers, an additional stool swab will also be collected. The accuracy of microbiome analysis resulting from perirectal swabs will be tested via comparison with analysis performed on stool specimens. The anal swab will be used to assess the presence of skin flora contamination in the peri-rectal swabs.

Spectrum of Stool Bacteria
For each enrolled research subject, the stool or perirectal swab collected at each visit will be used to identify any organisms present in the stool, including those identified in addition to the organisms of interest for the SCOUT study, E. coli and K. pneumoniae. These organisms that grow from SCOUT stool cultures are already identified as a result of the processing taking place for the SCOUT study. In this sub-study, the names of these other bacteria will be collected in a database to allow for a general description of the organisms that are present in children that are receiving antibiotic therapy for a urinary tract infection.

Outcome Measures

The primary outcome measure is:

Comparison of efficacy, based on symptomatic UTI as assessed up to or at the TOC visit (Day 11-14), between short-course and standard-course therapies.

The secondary outcome measures are:

Comparison of number of subjects that have a recurrent infection (includes a relapse UTI or a reinfection) at any time after the TOC visit (Day 11-14), following short-course versus standard-course (of antibiotics.

Comparison of the number of subjects that become colonized with antimicrobial resistant *Escherichia coli* (*E .coli*) and *Klebsiella pneumoniae (K. pneumoniae)* in the gastrointestinal tract as assessed through Day 24-30, following short-course versus standard course of antibiotics.

Comparison of the number of subjects with asymptomatic bacteriuria in subjects treated with short-course therapy as compared to the number of subjects treated with standard course therapy at the TOC visit

Comparison of the number of subjects with clinical symptoms that may be related to a UTI in subjects treated with short-course therapy as compared to the number of subjects treated with standard course therapy prior to or at the TOC visit.

Comparison of the number of subjects with positive urine culture prior to or at TOC between subjects treated with short-course therapy and those treated with standard course therapy.

The sub-study outcome measures are:

<u>Seattle Children's Hospital R21</u>
To determine if E. coli and K. pneumonia recovered from 2 or 3 stool cultures are more likely to be members of disease-associated subgroups within their respective species (E. coli phylogroups B2 and D, K. pneumoniae clusterKpI).

To determine if treatment-susceptible strains recovered from cultures during treatment (culture #1 for 5-day arm; cultures #1 or #2, 10-day arm) are more likely to be members of disease-associated subgroups.

To determine if treatment-resistant strains of either species are more likely than treatment susceptible strains to be recovered from cultures during treatment.

<u>Carbapenem resistant Enterobacteriaceae study</u>
To determine the overall prevalence of colonization with carbapenem resistant Enterobactericiae in children after completion of the course of antibiotic therapy for UTI

<u>Microbiome pilot study</u>
To determine the overall biodiversity of the gut microbial community, as measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To determine the overall fraction of gut microbiome community represented by gammaproteobacteria measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To determine the average ecological similarity between microbial communities within and between treatment groups at each follow-up following short-course and standard-course antibiotic exposure.

Spectrum of Stool Bacteria
To describe the bacteria present on the aerobic stool cultures obtained at each of the three study time points.

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

UTI

Urinary tract infections (UTIs) are among the most common bacterial infections encountered by primary care physicians. UTI is one of the most common serious bacterial infections during childhood [1]. Estimates of the cumulative incidence of UTI in children under age six years (three – seven percent in girls and one – two percent in boys) suggest that between 70,000 to 180,000 of the annual US birth cohort will have a UTI by age six [2].

Escherichia coli (E. coli) isolates account for 80-90% of all outpatient UTIs in both children and adults [10-12]. The remaining causes are other Gram-negative enterobacteria, especially *Klebsiella*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. *Klebsiella pneumoniae (K. pneumonia)* is a very important pathogen, both in its frequency of being associated with infection as well as in its tendency towards acquiring antimicrobial resistance.

Although antibiotics are the first treatment choice for urinary tract infections, antibiotic-resistant strains of *E. coli*, the most common cause of UTIs, are increasing worldwide. In many series, *K. pneumoniae* is an important cause of community acquired urinary tract infection in children and is an even more important cause of nosocomial infection in this population.

Emergence of Antimicrobial Resistance

The rapid emergence of antimicrobial resistance among bacteria is a major threat to the health of individuals as well as society as a whole. Infections with resistant bacteria increase morbidity and mortality, and greatly increase the financial burden of medical care [3-6]. While the number of resistant bacteria and the diversity of molecular mechanisms of resistance have increased sharply in recent years [7-8], the introduction of newer, effective antimicrobials has slowed significantly [9].

Antimicrobial resistance among uropathogens is increasing, especially among *E. coli* isolates [10-12]. *K. pneumoniae* account for a significant proportion of UTIs caused by non-*E. coli* organisms and resistant *K. pneumoniae* are increasingly prevalent and increasingly resistant to many antimicrobial agents including the broadest spectrum antimicrobial agents, the carbapenems [13-15]. The most significant change has been the rapid rise in prevalence of invasive infections caused by uropathogens resistant to

trimethoprim-sulfamethoxazole (TMP-SMX), ampicillin, and cephalosporins in both adults and children [10, 12, 16]. Of particular concern is the alarmingly high rate of resistance to third-generation cephalosporins in children admitted to the hospital for UTIs at some institutions [17]. Because these antimicrobial agents are commonly used as empiric therapy for UTI, emerging antimicrobial resistance has posed a significant challenge for clinicians. Timely and appropriate antibiotic therapy for UTIs in children, especially within 24 hours of onset of symptoms, reduces the risk of renal involvement. In addition, resistance to one class of antimicrobial agents used to treat UTIs has been associated with concurrent resistance to other antimicrobials [18]. For example, resistance to TMP-SMX has been associated with resistance to ampicillin, cephalothin, and third-generation cephalosporins [19-21]. Thus, the potential emergence of multi-drug resistant uropathogens is of great concern.

Table 1: Percent Isolates Susceptible to Each Antibiotic

ORGANISMS	Amikacin	Ampicillin	Amox/Clav	Amp/Sulb	Aztreonam	Cefazolin	Ceftazidime	Cefepime	Cefoxitin	Ceftriaxone	Cefuroxime	Ciprofloxacin	Gentamicin	Imipenem	Meropenem	Nitrofurant	Pip/Tazo	Tetracycline	Ticar/Clav	Tobramycin	Trimeth/Sulf
Escherichia coli																					
CHOP		54	78			93	99		96	99	93	92	94			96		80	89	97	78
PITT	99	52	82	96	87	96	97		96	93	92	95	99			79	96			94	79
Klebsiella pneumonia																					
CHOP		0	93			93	94		94	94	88	97	94			33		86	94	94	85
PITT	99	0	85		82	73	81	83		83	69	88	97			67	83			87	67

Evidence that Shorter Treatment Courses May Be Safe and Effective

Long-course antibiotic therapy for UTIs is intended to target the uropathogens, such as *E. coli*, but also alters the micro-ecology of the intestinal flora and its antibiotic resistance pattern, leading to prolonged colonization with antibiotic resistant *E. coli* that may persist for months [22]. This not only affects the individual child, but other community members as well. Studies have demonstrated that colonization with antimicrobial-resistant *E. coli* predisposes individuals who develop UTI to be infected with a resistant strain [23, 24]. Colonization with antimicrobial-resistant *E. coli* can be spread from the one child to the other within day care centers and from day care center contacts to household members [25-28]. Thus, children in day care represent a significant reservoir of potential resistant pathogens and day care attendance facilitates broad transmission throughout a community, expanding the numbers of individuals at risk of infection due to a resistant organism.

2.2 Rationale

The American Academy of Pediatrics (AAP) Practice Parameter for the treatment of UTI in infants and young children recommends a seven to 14-day antibiotic course, however most physicians routinely prescribe at least 10 days of antibiotic treatment. CHOP's Infectious Diseases research group recently published the results of a large cohort study of patients with UTI in 27 primary care pediatric practices and found that greater than 95% of UTIs were treated with a 10 to 14-day course of antibiotics [44]. Therefore, the large number of children with UTI and the duration of therapy in current standard practice make UTI an important target for reducing antibiotic exposure. Randomized clinical trials over the last 25 years have not provided definitive evidence supporting seven -14 day antibiotic courses for children with UTI. In fact, most studies showed no statistically significant difference in efficacy between short- and long-course antibiotic therapies. Although many of these studies were limited by small sample size, meta-analyses that pool the study results have shown similar results. Keren (a SCOUT co-investigator) and colleagues found that single dose or one-day of therapy was associated with a pooled relative risk of treatment failure of 2.73 (95%CI: 1.38-5.40) when compared to patients treated for seven -14 days [43]. However, for the subgroup of studies comparing three-day therapy to long-course therapy (seven-14 days), there was no increased risk of treatment failure (pooled RR 1.36 (95% CI: 0.69-2.72)). Tran, et al. in a meta-analysis of 1,279 children treated with either four days or less of therapy or with five days or more of therapy; they also noted no difference in the risk of treatment failure [44]. A single dose of amoxicillin was inadequate therapy for uncomplicated cystitis in children, however, three days of trimethoprim-sulfamethoxazole (TMP-SMX) was as effective as longer courses of therapy of TMP-SMX [44]. The Cochrane group conducted a systematic review comparing short course (two - four days) with standard duration (seven -14 days) of antimicrobial therapy for lower tract (e.g. cystitis) UTI in children [45]. Short-course antimicrobial therapy was found to be as effective as seven-14 days in eradicating lower tract UTI in children. In summary, meta-analyses suggest that treatment of UTI in children should not be less than three days, but that treatment with three or more days of therapy approaches treatment success of long-course therapy (seven -14 days).

Due to the frequency of UTIs in children, the duration of currently used antibiotic therapy, and the limitations of previous studies investigating the effectiveness of shorter-courses of therapy, there is a need for an adequately powered and well-designed trial to determine the efficacy and safety (e.g. incidence/severity of SAEs) of shorter course antibiotic therapy. Shortening antibiotic duration for UTIs and other infections reduces antibiotic exposure and should decrease the development of antimicrobial resistance.

Rationale for Studying Urinary Tract Infections in Children

SCOUT will evaluate shorter courses of antibiotics for UTIs in children because:
(1) UTIs are common;
(2) UTIs are currently treated with a long course of antibiotics (10-14 days);
(3) There is evidence that shorter treatment courses may be safe and effective; and
(4) Antibiotic resistance among uropathogens (e.g. *E. coli* and *K. pneumoniae*) has a significant clinical and public health impact.

Rationale for Shorter Antimicrobial Treatment Duration

A commonly proposed strategy to reduce antimicrobial resistance is to limit antimicrobial use to the minimum duration necessary for safe and effective treatment [14]. Decreasing the duration of antimicrobial therapy reduces antimicrobial selection pressure on colonizing and environmental flora, which should decrease their acquisition of antimicrobial resistance. Antibiotic pressure, which promotes the exchange of resistance genes by various transfer mechanisms, has been proven to be the single most important factor predisposing patients to infection with resistant organisms [29]. On a population level there is a clear relationship between total antibiotic consumption and resistance rates among bacterial pathogens [30-32]. Multiple clinical studies have documented an association between prolonged courses of therapy and the development of resistance in individual patients during therapy [33-35]. In fact, antimicrobial exposure is one of the most common risk factors for colonization and infection with antibiotic-resistant organisms [35].

The basis for currently prescribed lengths of antibiotic therapy for most infectious diseases is largely historical, and not evidence-based. Because of the lack of data regarding the minimum effective duration of therapy, physicians often extend antimicrobial courses well beyond the time of clinical improvement. However, shorter courses of therapy have been shown to be effective in treating several infectious diseases, including traveler's diarrhea, community-acquired pneumonia, ventilator-associated pneumonia, cystitis in adults, acute otitis media, pharyngitis, and cellulitis [34, 36-42].

Antibiotic Resistance among Uropathogens (e.g. *E. coli* and *K. pneumoniae*) has a Significant Clinical and Public Health Impact

Antimicrobial resistance among uropathogens is increasing, especially among *E. coli* isolates, which account for 80-90% of all outpatient UTIs in both children and adults [10-12]. *K. pneumoniae* account for a significant proportion of UTIs caused by non-*E. coli* organisms and resistant *K. pneumoniae* are increasingly prevalent and increasingly resistant to many antimicrobial agents including the broadest spectrum antimicrobial agents, the carbapenems [40-42]. The most significant change has been the rapid rise in

prevalence of invasive infections caused by uropathogens resistant to TMP-SMX, ampicillin, and cephalosporins in both adults and children [10,12,16]. Of particular concern is the alarmingly high rate of resistance to third-generation cephalosporins in children admitted to the hospital for UTIs at some institutions [17]. Because these antimicrobial agents are commonly used as empiric therapy for UTI, emerging antimicrobial resistance poses a significant challenge for clinicians. Timely and appropriate antibiotic therapy for UTI in children, especially within 24 hours of onset of symptoms, reduces the risk of renal involvement [18]. In addition, resistance to one class of antimicrobial agents used to treat UTI has been associated with concurrent resistance to other antimicrobials. For example, resistance to TMP-SMX has been associated with resistance to ampicillin, cephalothin, and third-generation cephalosporins [19-21]. Thus, the emergence of multi-drug resistant uropathogens is of great concern.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Short-course Antimicrobial Therapy Arm:

Five Days of Antibiotics + Five Days of the Placebo

Five days of antibiotic therapy may be less effective than 10 days, and so children in the short-course five-day arm may have a higher risk of treatment failure. The risk is mitigated by the fact that only those children that have shown clinical improvement (afebrile and asymptomatic) after initiation of antibiotic therapy will be enrolled in the study. This is one reason why subjects are enrolled following initiation of antibiotic therapy, rather than prior to initiation.

Standard-of-Care Antimicrobial Therapy Arm: 10 Days of Antibiotics

Long-course antibiotic therapy for UTI is intended to target the uropathogens, such as *E. coli* and *K. pneumoniae*, but also alters the micro-ecology of the intestinal flora and its antibiotic resistance pattern, leading to prolonged colonization with antibiotic resistant *E. coli* and *K. pneumoniae* that may persist for months [22]. This not only affects the individual child, but other community members as well. Studies have demonstrated that colonization with antimicrobial-resistant *E. coli* and *K. pneumoniae* predisposes individuals who develop UTI to be infected with a resistant strain [23-24].

Children on the continued antibiotic treatment arm may have a higher likelihood of developing resistant bacteria than children on the placebo arm; however, their risk will be no greater than what occurs in standard clinical practice.

Drug Safety

The antibiotics used in this study (trimethoprim-sulfamethoxazole (TMP-SMX) or cefixime or cephalexin) are not investigational drugs and are already used in current clinical practice to treat UTIs. Subjects on the continued antibiotic arm will receive the same antibiotic initially prescribed to them that they would have completed if not part of the SCOUT clinical study. Note: subjects initially prescribed cefdinir will receive cefixime or cefixime placebo.

Trimethoprim-sulfamethoxazole (TMP-SMX), cefixime, cefdinir, and cephalexin have been used to treat UTIs in children for decades. These four antibiotics have established safety records for pediatric use and have well described side effect profiles. Antibiotics disturb the normal bowel bacterial flora. During study visits and the follow-up phone call, study participants will be asked to report any potential side effects of the study product, any residual UTI symptoms, and if the child had any hospital or doctor visits for UTI symptoms since their last study contact, and on any possible adverse reactions. All potential serious adverse events (SAEs) or reported side effects will be recorded as study data.

Although the study is using four FDA-approved antibiotics, this study will be conducted under an IND because it will be used to inform the medical community regarding alternative treatment regimens for the antibiotic treatment duration.

Trimethoprim-Sulfamethoxazole (TMP-SMX)

TMP-SMX may cause allergic reactions that range from a mild (skin rash) to severe (Stevens - Johnson syndrome), although severe reactions are extremely rare. Other potential side effects of TMP-SMX include sun sensitivity, recurrent vaginitis, granulocytopenia, and dizziness. Families will be queried about a subject's history of allergies to TMP-SMX. Families will be provided with literature on adverse reactions to TMP-SMX and instructions to call study staff if any evidence of such a reaction occurs.

Cefixime

Cefixime should be avoided by children with a known allergy to cephalosporin type antibiotics. Cefixime is generally well tolerated and side effects are usually transient. Cefixime may cause gastrointestinal issues such as diarrhea, gas, and stomach pain, as well as more severe events such as bloody stools, severe nausea or vomiting or allergic reactions. Families will be queried about a subject's history of allergies to cefixime. Families will be provided with literature on adverse reactions to cefixime and instructions to call study staff if any evidence of such a reaction occurs.

Cefdinir

On occasion cefixime may not be available to the prescribing primary care physician. Under such circumstances it is likely that cefdinir will be prescribed as an alternative agent to cefixime instead. The two antibiotics have therapeutic equivalence.
If a subject prescribed cefdinir is enrolled and randomized they will be given either five additional days of cefixime (standard-of-care) or the cefixime placebo (short-course) for the day six – ten SCOUT Study period. Families will be queried about a subject's history of allergies to cefixime. Families will be provided with literature on adverse reactions to cefixime and instructions to call study staff if any evidence of such a reaction occurs.

Cephalexin

Cephalexin should be avoided by children with a known allergy to penicillin or cephalosporin type antibiotics. Allergic reactions in the form of rash, urticaria, angioedema, and, rarely, erythema multiforme, Stevens-Johnson syndrome, or toxic epidermal necrolysis have been observed. These reactions usually subsided upon discontinuation of the drug. Cephalexin may cause gastrointestinal issues including pseudomembranous colitis, diarrhea, dyspepsia, gastritis, and abdominal pain. Families will be provided with literature on adverse reactions to cefixime and instructions to call study staff if any evidence of such a reaction occurs.

Risks Associated with Catheterization

If a clean-catch sample cannot be obtained in a symptomatic subject a catheter obtained sample should be obtained.

Catheterization will be performed as a sterile medical procedure and will only be done by trained, qualified clinicians, using equipment designed for infants and young children. If correct technique is not used, there may be risks of urinary catheterization including:

- injury to the urethra and prostate (male) caused by traumatic insertion of the catheter;
- narrowing of the urethra due to scar tissue caused by the insertion of a catheter; and
- injury to the bladder caused by incorrect insertion of the catheter;
- catheter associated urinary tract infection;
- pain and discomfort;
- paraphimosis in an uncircumcised male.

The SCOUT Study Nurses/Nurse Practitioners/Physicians will take all possible precautions to reduce the risk of infection. Such precautions include:

- Cleansing the urethral area (area where catheter exits body) and using a sterile catheter;
- The catheter will be lubricated with a water-soluble sterile lubricant prior to insertion;
- Disconnecting drainage bag from catheter only with clean hands; and
- Use of a thin (age and size appropriate) catheter to reduce risk of harming the urethra during insertion.

These potential risks will be shared with the child's family and outlined in the informed consent form.

Risks Associated with Rectal Swab

Performing a rectal swab for the collection of stool sample has almost no risk. Some parents and children find it aesthetically unpleasant. For some older children, it may be psychologically distressing.

Potential Loss of Confidentiality

SCOUT will institute strict procedures to maintain confidentiality. No personal information, such as names, contact information, social security numbers, etc. will be stored in the study database managed by the CHOP-Westat BDMC. Information such as dates of birth and dates of events will be stored in the database by study ID number. Physical files linking study ID numbers to personal information will reside in locked files in the office of the Principal Investigator or the Study Coordinator at the clinical site. Any publications or presentations resulting from this work will not identify participants by name, but will only present aggregate data. Our prior research employing similar precautions has demonstrated that these techniques are very successful in assuring the protection of subjects. Genetic testing is not planned; however, there may be future bacterial genomic testing to determine the mechanism of resistance and epidemiological relationships.

Protection against Risks to Study Personnel

The only potential hazard to study personnel is exposure to feces and urine when the Study Nurse/Nurse Practitioner/Physician collects the stool and urine sample. All the study personnel that may be exposed will be trained in safety precautions.

Study staff will be trained according to the International Air Transport Association (IATA) requirements before shipping stool samples to the PITT IDRL.

2.3.2 Potential Benefits

There is the possibility of direct benefit to the subject enrolled in this study. Study subjects in the short course arm may experience decrease antibiotic side effects and a reduction in opportunistic or secondary infections such as vaginitis.

Also, information obtained from this study may show if shortening antibiotic duration for UTI decreases the development of antimicrobial resistance as well as help preserve the effectiveness of existing antimicrobials. The results of this study will assist health-care providers in making decisions regarding the use of antimicrobial therapy for treating children with UTIs, and may benefit the health of the general community by slowing the evolution of antimicrobial resistance. By reducing the occurrence of resistant organisms, society benefits through easier treatment of this common infection. Careful follow-up will allow for early termination of study participation in the case of children categorized as treatment failures.

The SCOUT Study will provide data to guide the future effective management of pediatric UTIs using strategies aimed to control the emergence of antimicrobial resistance among bacteria.

3 DEFINITIONS

Fever/Febrile: A documented temperature of at least 100.4° F or 38° C, measured anywhere on the body (oral, axillary, tympanic, or rectal).

Afebrile: NO documented temperature greater than or equal to 100.4 °F or 38°C, measured anywhere on the body (oral, axillary, tympanic, or rectal) in the 24 hours prior to the enrollment visit.

Urinary Tract Infection:

I. The presence (in the medical record or by parent report) of at least one of the symptoms consistent with the diagnosis of UTI including:

- Symptoms for all children (ages two months to 10 years):
  - fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
  - dysuria
- Additional symptoms for children > 2 years of age:
  - suprapubic, abdominal, or flank pain or tenderness OR
  - urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)
- Additional symptoms for children ≥ 2 months to 2 years of age:
  - poor feeding OR
  - vomiting

AND

II. Pyuria on urinalysis

- ≥10 WBC/mm^3 (uncentrifuged specimen) OR
- ≥5 WBC/hpf (centrifuged specimen), OR
- Leukocyte esterase ≥ trace on dipstick.

AND

III. Culture proven infection with a single uropathogen:

- ≥5 x 10^4 CFU/mL (catheterized or suprapubic aspiration urine specimen) OR
- ≥10^5 CFU/mL (clean void specimen).

Prescription medication: Medication prescribed by an authorized/licensed clinician.

Study product: The term 'study product' refers to both the study antibiotic (TMP-SMX, cefixime, and cephalexin) and the corresponding placebo.

Clinical Improvement at Randomization:

1) Afebrile: No documented temperature equal or greater than 100.4 °F or 38°C, measured anywhere on the body (oral, axillary, tympanic, or rectal) in the 24 hours prior to the Enrollment Visit
   AND

2) Asymptomatic: Report NONE of the following symptoms related to UTI:

- Symptoms for all children (ages two months to 10 years):
  - fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
  - dysuria
- Additional symptoms for children > 2 years of age:
  - suprapubic, abdominal, or flank pain or tenderness OR
  - urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)
- Additional symptoms for children ≥ 2 months to 2 years of age:
  - poor feeding OR
  - vomiting

Treatment Failure:
A subject will be categorized as a treatment failure, if he/she has a symptomatic UTI in period between Day 6 through the Day 11 – 14 Test of Cure (TOC) Visit: (See Appendix B)

- Symptoms for all children (ages two months to 10 years):
  - fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
  - dysuria
- Additional symptoms for children > 2 years of age:
  - suprapubic, abdominal, or flank pain or tenderness OR
  - urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)
- Additional symptoms for children ≥ 2 months to 2 years of age:
  - poor feeding OR
  - vomiting

AND

I. Pyuria on urinalysis

- $\geq$10 WBC/mm^3 (uncentrifuged specimen) OR
- $\geq$5 WBC/hpf (centrifuged specimen), OR

Leukocyte esterase $\geq$ trace on dipstick.

AND

II. Culture proven infection with a single uropathogen:

- $\geq 5 \times 10^4$ CFU/mL (catheterized or suprapubic aspiration urine specimen) OR
- $\geq 10^5$ CFU/mL (clean void specimen).

NOTE: As per the above criteria, asymptomatic subjects (including subjects assessed as having asymptomatic bacteriuria) at the Day 11-14 TOC visit will NOT be considered a treatment failure for the primary outcome measure.

Pyuria on urinalysis:

- ≥10 WBC/mm^3 (uncentrifuged specimen) OR
- ≥5 WBC/hpf (centrifuged specimen), OR
- Leukocyte esterase ≥ trace on dipstick.

Recurrent Infection: A UTI that occurs anytime after the Day 11 – 14 Test of Cure Visit. This can include a relapse infection or reinfection. (See Appendix B)

Asymptomatic Bacteriuria:
Asymptomatic Bacteriuria is defined in any SCOUT subject by:

(1) Absence of symptoms attributable to UTI including fever AND/OR the following:
- Symptoms for all children (ages two months to 10 years):
  - fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
  - dysuria
- Additional symptoms for children > 2 years of age:
  - suprapubic, abdominal, or flank pain or tenderness OR
  - urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)
- Additional symptoms for children ≥ 2 months to 2 years of age:
  - poor feeding OR
  - vomiting

AND

(2) A positive urine culture with a single uropathogen:
- ≥ 5 x 10^4 CFU/mL (catheterized or suprapubic aspiration urine specimen) OR
- >10^5 CFU/mL (clean void specimen).

Diarrhea: Three loose, watery stools that occur in one day or two or more loose, watery stools in two consecutive days.

Antibiotic Resistance: Resistance against amoxicillin-clavulanate, TMP-SMX or evidence of ESBL production.

Multiple Drug Antibiotic Resistance: Resistance to two or more agents (this includes resistance against amoxicillin and amoxicillin-clavulanate).

***K. pneumoniae* Multiple Drug Antibiotic Resistance**: Resistance to either two or more of the four agents (TMP-SMX, amoxicillin-clavulanate, cefixime or cephalexin) that the SCOUT Study will test (excluding amoxicillin resistance). Those initially prescribed cefdinir are switched to bioequivalent cefixime.

4 OBJECTIVES

4.1 Study Objectives

The primary and secondary objectives of this study are as follows:

Primary Objective:

To determine if halting antimicrobial therapy in subjects who have exhibited clinical improvement 5 days after starting antibiotic therapy (short course therapy) have the same failure rate (symptomatic UTI) through TOC (visit Day 11-14) as subjects who continue to take antibiotics for an additional 5 days (standard course therapy).

Secondary Objectives:

To determine if short-course therapy compared to standard course therapy results in similar numbers of children experiencing a recurrent infection (relapse and reinfection).

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with asymptomatic bacteriuria.

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with gastrointestinal colonization of antimicrobial resistant *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*).

To determine if short-course therapy compared to standard course therapy results in similar numbers of subjects presenting with clinical symptoms that may be related to UTI.

To determine if short-course therapy compared to standard course therapy results in similar numbers of subjects with positive urine cultures up to and including TOC.

Sub-Study Objectives:

Seattle Children's Hospital R21
To determine if E. coli and K. pneumonia recovered from 2 or 3 stool cultures are more likely to be members of disease-associated subgroups within their respective species (E. coli phylogroups B2 and D, K. pneumoniae clusterKpI).

To determine if treatment-susceptible strains recovered from cultures during treatment (culture #1 for 5-day arm; cultures #1 or #2, 10-day arm) are more likely to be members of disease-associated subgroups.

To determine if treatment-resistant strains of either species are more likely than treatment susceptible strains to be recovered from cultures during treatment.

Carbapenem resistant Enterobacteriaceae study
To determine the overall prevalence of colonization with carbapenem resistant Enterobactericiae in children after completion of the course of antibiotic therapy for UTI

Microbiome pilot study
To determine the overall biodiversity of the gut microbial community, as measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To determine the overall fraction of gut microbiome community represented by gammaproteobacteria measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To determine the average ecological similarity between microbial communities within and between treatment groups at each follow-up following short-course and standard-course antibiotic exposure.

Spectrum of Stool Bacteria
To describe the breadth of bacteria present in the stool of children receiving therapy for a urinary tract infection. These data will allow for comparison of aerobic bacteria composition in these children and present an opportunity to contrast them to similar data available from other pediatric patient populations.

4.2 Study Outcome Measures

In order to capture all cases of UTI, and to enhance our ability to identify any symptom related to the urinary tract, we modeled when to have our follow up study visits and phone call after the FDA's Guidance for Complicated and Uncomplicated Urinary Tract Infections [51,52]. We will have the:

1) Test-of-Cure Post-Treatment (TOC) Visit on Day 11 -14 (FDA recommendation: five - nine days after completing the study product); in order to evaluate the primary endpoint of symptomatic UTI. Secondary endpoints of asymptomatic bacteriuria, positive urine culture, and presentation of clinical symptoms that may be related to UTI will also be assessed.
2) Outcome Assessment Visit on Day 24 – 30 (14 – 20 days after completing the study product) in order to evaluate the secondary endpoints: recurrent infection (includes a relapse UTI or a reinfection) at any time after the TOC visit (Day 11-14); colonization with antimicrobial resistant *E. coli* and *K. pneumoniae* in the gastrointestinal tract; and
3) Follow-up call on Day 38 – 44 (FDA: 28-34 days after completing the study product). Safety follow-up during which subjects will be asked regarding presence or absence of UTI symptoms, if they sought medical care for possible recurrence of UTI, and if they experienced any inter-current illnesses.

4.2.1 Primary and Secondary Outcome Measures

The primary outcome measure is:

- Comparison of efficacy based on symptomatic UTI as assessed at the TOC visit (Day 11-14), between short-course and standard-course of antibiotics.

Since the primary objective of the study is to establish that short-course therapy is as effective as the standard therapy, the analysis of the primary endpoint will be based on a non-inferiority test. The primary analysis will be done on the intent to treat (ITT) population.

The secondary outcome measures are:

- Comparison of the number of subjects that have a recurrent infection (includes a relapse UTI or a reinfection) at any time after the TOC visit (Day 11-14), following short-course versus standard-course of antibiotics.
- Comparison of the number of subjects that become colonized with antimicrobial resistant *Escherichia coli* (*E .coli*) and *Klebsiella pneumoniae (K. pneumoniae)* in the gastrointestinal tract as assessed through Day 24-30, following short-course versus standard course of antibiotics.
- Comparison of the number of subjects with asymptomatic bacteriuria at the TOC visit, following short-course versus standard-course of antibiotics.

- Comparison of the number of subjects with clinical symptoms that may be related to a UTI prior to or at the TOC visit, following short-course versus standard-course of antibiotics.
- Comparison of the number of subjects with positive urine cultures prior to or at the TOC visit, following short-course versus standard-course of antibiotics.

For a summary of individual subject study outcomes against the primary outcome measure see Table 2.

Sub-study Outcome Measures:
Seattle Children's Hospital R21

- Presence of E. coli, presence of K. pneumoniae
- Sub-types of E. coli (0=phylogroups A or B1, 1=phylogroups B2 or D), sub-types of K. pneumoniae
- Presence of treatment-susceptible E. coli, presence of treatment-susceptible K. pneumoniae
- Presence of treatment-resistant E. coli, presence of treatment-resistant K. pneumoniae

Carbapenem resistant Enterobacteriaceae study

- Prevalence of colonization with carbapenem resistant *Enterobactericiae* in children after completion of the course of antibiotic therapy for UTI

Microbiome pilot study

- Comparison of the overall biodiversity of the gut microbial community, as measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.
- Comparison of overall fraction of gut microbiome community represented by gammaproteobacteria measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.
- Comparison of average ecological similarity between microbial communities within and between treatment groups at each follow-up following short-course and standard-course antibiotic exposure.

Spectrum of Stool Bacteria

- Description of the bacteria present on the aerobic stool cultures obtained at each the three study time points.

Table 2: SCOUT Study Possible Outcomes Day 6-44.

Outcome scenario	UTI Symptomology		Urine collection method	Urine culture result		Pyuria present		Composite Outcome/Follow-up	Repeat urine culture result/outcome	
	Symp	Asymp[1]		+	-	Yes	NO			
1	X		Clean Catch or Catheter	X		X		Treatment Failure		
2	X		Clean Catch or Catheter	X			X	No UTI		
3	X		Clean Catch or Catheter		X	X		Follow-up required. Contact subject in 24-48 hrs. Perform repeat urine culture if still symptomatic.	+ Treatment Failure[1]	- No UTI
4	X		Clean Catch or Catheter		X		X	Follow-up required. Contact subject in 24-48 hrs - Perform repeat urine culture if still symptomatic.	+ No UTI (since no Pyuria)	- No UTI
5a		X	Clean Catch	X		X		No UTI (Asymptomatic Bacteriuria)		
5b		X	Bag	X		X		No UTI (Asymptomatic Bacteriuria)		
6a		X	Clean Catch	X			X	No UTI (Asymptomatic Bacteriuria)		
6b		X	Bag	X			X	No UTI (Asymptomatic Bacteriuria)		

7		X	Clean Catch or Bag		X	X		No UTI		
8		X	Clean Catch or Bag		X		X	No UTI		

[1] If a urine sample cannot be obtained from asymptomatic subjects at the TOC visit, another attempt will be made to obtain a sample at the Day 24-30 Outcome Assessment Visit. These subjects are evaluable for the primary endpoint measure (considered a success); however, they will not be evaluable for the asymptomatic bacteriuria or urine culture secondary endpoints.

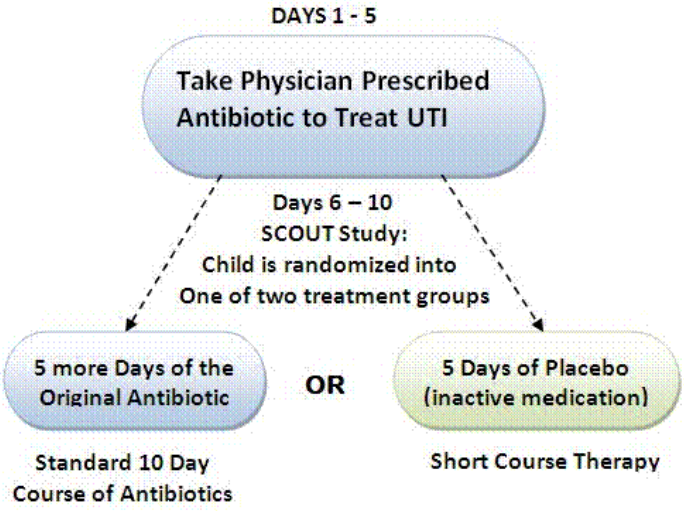
Note: Urine collection in children that are toilet trained will be by clean catch. For infants and young children (prior to being toilet trained) the initial urine sample will be obtained by bag method (if asymptomatic), clean-catch if symptomatic (or catherization if a sample cannot be obtained by clean-catch). Also see Section 9.2.3.1 for listing of urine collection by age group/symptomology.

5 STUDY DESIGN

The SCOUT Study is a multi-center, centrally randomized, double-blind, placebo-controlled non-inferiority clinical trial. Children will be stratified based on the presence of fever at initial presentation of UTI symptoms and by specific antibiotic therapy prescribed by the original treating clinical provider. Children who have demonstrated clinical improvement five days after starting the originally prescribed antibiotic (afebrile and asymptomatic) will be randomized to standard arm or the short-course arm. Subjects enrolled prior to completion of Day 5 of their originally prescribed antibiotic treatment, will be instructed to complete Day 5 dosing prior to initiating SCOUT study product on Day 6. Subjects enrolled Day 6 should not have started the Day 6 of their originally prescribed antibiotic treatment. The SCOUT study product will consist of trimethoprim-sulfamethoxazole sulfamethoxazole (TMP-SMX), cefixime, cephalexin and the corresponding placebos. If a child initially prescribed cefdinir is enrolled, they will be randomized to either five additional days of cefixime or the cefixime placebo.

About 746 subjects will be enrolled in order to reach 672 evaluable subjects, but the total number that will be 'enrolled / consented' may exceed 746 if the study has not enrolled 672 evaluable for the primary endpoint.

Figure 1: Randomization



5.1 Sub-studies

Seattle Children's Hospital R21

An R21-funded sub-study titled, "Differences in infecting and colonizing Enterobacteriaceae from short-course vs. standard therapy of pediatric UTI" is currently planned with collaborators at Seattle Children's Hospital. This sub-study hypothesizes that both the antibiotic agent and the length of therapy will affect the patients' indigenous flora and thus affect both the likelihood of recovering *E. coli* and/or *K. pneumoniae* from stool and the resistance phenotypes of the recovered isolates. The sub-study will utilize PCR- and sequence-based methods to characterize phylogenetic and resistance properties of the recovered isolates. The sub-study will also test the hypothesis that (1) *E. coli* and *K. pneumonia* recovered from 2 or 3 stool cultures are more likely to be members of disease-associated subgroups within their respective species (*E. coli* phylogroups B2 and D, *K.* pneumoniae cluster KpI); (2) treatment-susceptible strains recovered from cultures during treatment (culture #1 for 5-day arm; cultures #1 or #2, 10-day arm) are more likely to be members of disease-associated subgroups; (3A) treatment-resistant strains of either species are more likely than treatment susceptible strains to be recovered from cultures during treatment; and (3B) treatment susceptible strains of either species are more likely to be recovered from cultures during treatment in the AMC arm than in the TMP/SMX or CFX/CFD arms.

Carbapenem resistant enterobacteriaceae

For each enrolled research subject, the stool or perirectal swab collected at the Test of Cure visit will also be utilized to perform additional screening for the presence of carbapenem resistant enterobacteriaceae. The results of this additional screening will be used to determine the prevalence of colonization with carbapenem-resistant Enterobacteriaceae (overall and for specific pathogens) in ambulatory children after completion of treatment for UTI.

Microbiome pilot study

For each subject at CHOP who agrees to participate in the Microbiome pilot study, an additional perirectal swab and an anal swab will be collected. Subjects in diapers will also have a third additional stool swab collected if a soiled diaper can be obtained. The additional swabs will be collected at each visit and will be used to determine the composition of the subjects' microbiome. Microbiome composition will be quantified using high-throughput DNA amplicon sequencing of 16S ribosomal RNA genes. The accuracy of microbiome analysis resulting from perirectal swabs will be tested via comparison with analysis performed on stool specimens. All samples will be shipped immediately or frozen overnight prior to shipment to collaborators at the University of Minnesota. Additionally, subjects agreeing to participate will be asked to complete a questionnaire providing data about feeding (breast milk, formula, timing of introduction of solid foods); most recent antibiotic prescriptions (name, dose, and duration); and number of antibiotic courses in life.

Spectrum of Stool Bacteria

For each enrolled research subject, the stool or perirectal swab collected at each visit will be used to identify any organisms present in the stool in addition to the organisms of interest for the SCOUT study, E. coli and K. pneumoniae. These organisms that grow from SCOUT stool cultures are already identified as a result of the processing taking place for the SCOUT study. In

this sub-study, the names of these other bacteria will be collected in a database to allow for a general description of the organisms that are present in children that are receiving antibiotic therapy for a urinary tract infection.

6 STUDY ENROLLMENT AND WITHDRAWAL

The two participating clinical trial centers, CHOP and PITT will enroll subjects over an approximately four and a half year enrollment period until 672 subjects have been enrolled that are evaluable for the primary outcome measure.

6.1 Subject Inclusion Criteria: Must Meet All

1) Age at randomization: at least two months (at least 36 weeks gestational age for subjects < two years of age) to 10 years of age (120 months)

2) Confirmed UTI diagnosis (see definition Section 3)

3) Documented Clinical Improvement at Randomization

   a. Afebrile : No documented temperature equal or greater than 100.4 °F or 38°C (measured anywhere on the body) 24 hours prior to the enrollment visit

   AND

   b. Asymptomatic: report NONE of the following symptoms:

• Symptoms for all children (ages two months to 10 years):
- fever (a documented temperature of at least 100.4 °F OR 38°C measured anywhere on the body)
- dysuria

• Additional symptoms for children > 2 years of age:
- suprapubic, abdominal, or flank pain or tenderness OR
- urinary urgency, frequency, or hesitancy (defined as an increase in these symptoms from pre-diagnosis conditions)

• Additional symptoms for children ≥ 2 months to 2 years of age:
- poor feeding OR
- vomiting

4) Only children who have been prescribed one of the four antibiotics for which a placebo is available will be eligible to participate.

   1 TMP-SMX

   2 Cefixime

   3 Cefdinir

   4 Cephalexin

   (Note a child that received a one-time dose of I.M. or I.V. medication (i.e. in ER or clinic) prior to starting on the one of the four oral medications is eligible for enrollment).

5) Parental/guardian permission (informed consent) and if appropriate, child assent (if ≥ seven years of age).

6.2 Subject Exclusion Criteria: Meets at Least One

1) A urine culture proven infection with a second uropathogen > 10,000 CFU/mL collected via suprapubic aspiration or catheter or > 50,000 CFU/mL collected via clean void
2) A child hospitalized with a UTI that has the following: concomitant bacteremia associated with the UTI, urosepsis, or is in intensive care.
3) A child whose urine culture reveals an organism that is resistant to the initially prescribed antibiotic.
4) A child with a catheter-associated UTI.
5) A child with known anaphylactic allergies to the study products.
6) A child with phenylketonuria (PKU)
7) A child diagnosed with congenital anomalies of the genitourinary tract
8) UTI in children with known anatomic abnormalities of the genitourinary tract other than VUR, duplicated collection systems, and hydronephrosis.
9) A child that is not able to take oral medications
10) Previous surgery of the genitourinary tract (except circumcision in male children)
11) Presence of an immunocompromising condition (e.g., HIV, malignancy, solid-organ transplant recipients, use of chronic corticosteroids or other immunosuppressive agents).
12) Unlikely to complete follow-up (e.g. not available for the two follow-up study visits and the follow-up phone call)
13) A child with a known history of type I hypersensitivity of the study antibiotics to be prescribed
14) Enrollment in another antibiotic study less than 30 days prior to enrollment visit.
15) Previous enrollment of individuals in this study.
16) Planned enrollment during this study coincides with enrollment in another therapeutic drug study (excluding vaccine).
17) A child with a history of UTI within the past 30 days
18) A child with known Grade III-V VUR

19) A child taking antibiotic prophylaxis for any reason.

20) A child who has started Day 6 of the originally prescribed antibiotic treatment.

6.3 Recruitment Strategy

Children will be recruited from three different sources: primary care sites, general pediatrics inpatient units and the emergency departments from the following locations:

CHOP

- CHOP's Inpatient Service;
- CHOP's outpatient practice and urgent care network, which spans two states (Pennsylvania and New Jersey);
- The CHOP Emergency Department; and
- The General Pediatrics Inpatient Unit of CHOP.

PITT

- PITT's Primary Care Centers;
- PITT's Practice Based Research Network, PittNet;
- The PITT Emergency Department; and
- The Inpatient Service at PITT.

At both CHOP and PITT, SCOUT Study informational pamphlets will be prepared and distributed to all recruiting locations. These materials will explain the study, the criteria for inclusion and exclusion, and the study procedures and timeframe. These pamphlets can be used to familiarize primary care, ED and inpatient unit clinicians as well as subjects and families with the goals of the study. In addition, to further aid recruitment, a study webpage will be available for additional information for clinicians and subject families.

6.4 Treatment Assignment Procedures

6.4.1 Randomization Procedures

The SCOUT Study is a randomized, double-blind, placebo-controlled clinical trial.

After a potential subject has been determined to meet eligibility criteria, the subject will be given a random treatment assignment of study product to either short-course or standard-of-care therapy. Randomization will be done using fixed blocks of size four. Subjects will be stratified (within site) by febrile/non-febrile condition when originally diagnosed and antibiotic prescribed at initiation of therapy. That is, separate randomization sequences will be generated for each site for each of eight strata defined

by febrile/non-febrile condition and the four initially prescribed possible antibiotics. Treatment kits will be pre-packaged and randomized to active or placebo in a 1:1 ratio. Subjects initially prescribed cefdinir will be randomized to either cefixime or cefixime placebo.

The SCOUT Study will employ WesTraxTM an interactive voice response (IVR) system to randomize subjects. This system uses the telephone as a means to input data. Pre-recorded prompts are played listing the various options available to the user or requesting responses to particular questions. Data are entered using the telephone touch-tone keypad and are written to the underlying databases. The Study Nurse/Nurse Practitioner/Physician will randomize the subject based on the responses collected during the call into the system at the Enrollment Visit.

The system security is enforced through the recognition of unique User ID and PIN numbers assigned to each system user. Each PIN number is associated with a list of system privileges.

The Westat staff will train site personnel prior to study enrollment. In addition, at the time of site activation, each user at the activated site will receive an instruction packet. User instructions are project-specific and the packet will contain the necessary instructions for the user's appropriate system access level.

The WesTrax^{TM} system will be available to all participating centers, 24 hours a day, seven days a week, with allowances for system maintenance operations, for the duration of study enrollment. If users have questions about the system, a WesTrax coordinator is on call 24 hours a day, seven days a week and can be reached by dialing the Help Center. All calls requiring action are recorded in a help desk database and metrics are readily available on calls for evaluation of additional training tool needs.

6.4.2 Masking Procedures

The study subjects and their families, investigators, and SCOUT Study team staff will remain blinded / masked to study treatment assignment (five more days of antibiotics therapy or five days of placebo) throughout the study. The subjects and their families, investigators, and SCOUT Study team staff will not be blinded to which one of the four antibiotics the subject was originally prescribed.

The study products and placebo will be prepared centrally, by the UPENN Investigational Pharmacy, for both CHOP and PITT. Only the Penn IDS Pharmacist will be aware of the study product bottle assignments. The staff at the medication storage areas at CHOP and PITT will be responsible for dispensing coded study products to preserve study blinding. For participants randomized to standard-of-care therapy, the pharmacy will provide the same medication prescribed to initially treat the UTI (NOTE: subjects initially prescribed cefdinir will be provided cefixime or cefixime placebo). The

Penn IDS pharmacy will prepare placebos that resemble the taste and consistency of the active antimicrobials. All study product kits will be packaged with an identical appearance.

The UPENN Investigational Pharmacy will maintain study product accountability records for both CHOP and PITT. The study product kits will be similarly labeled with a numerical code that masks site investigators, site staff, parent(s)/guardian(s) and children to the formulation. In addition, the UPENN Investigational Pharmacy will place a two-part label on each of the study product kits. One part of the label will be removed and placed on the subject's case report form or the medication accountability log and the second part of the label will remain on the bottle. The Penn IDS pharmacist will be the only person to perform the unmasking if needed.

During the consenting process it will be explained to the parents of any potential enrollees that the study product (treatment or placebo) that will be provided after day five may or may not taste exactly the same as the originally prescribed medication, and look and smell slightly different because it will be provided to the study pharmacy from a manufacturer which may be different than the manufacturer who provide the medication for the first five days.

6.4.3 Reasons for Withdrawal

The SCOUT protocol has been developed to minimize the number of participants lost to follow-up and reduce barriers to participation. The schedule of contacts is designed to facilitate participant retention through regular contact in a short timeframe.

Participation in the SCOUT Study is voluntary. Subjects and their families can decide to discontinue participation at any time for any reason during the study by notifying study staff.

In addition, the Investigators may discontinue participation for any subject at their discretion: if in their professional opinion, the subject's health, safety, and/or well-being is threatened by continued participation in the study or they may withdraw those subjects that do not comply with the study, or to protect the subject for safety reasons or for administrative reasons. Subjects may also be discontinued from the study at the Investigator's discretion for lack of adherence to study treatment or visit schedules, AEs, loss to follow-up or a protocol violation.

Symptomatic subjects that develop a positive urine culture during the study period will not be considered withdrawn from the study, they will continue to be seen/contacted for any remaining study visits as part of safety follow-up. These subjects will be treated outside this protocol by a physician not associated with the SCOUT Study per current

standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician.

Subjects that develop a fever or worsening of symptoms after enrollment but prior to initiation of protocol treatment will be withdrawn from the study and considered entry failures. These subjects will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician.

It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events (AEs) after the subject completes or withdraws from the study, it will be recorded in the source documents and on the CRF.

6.4.4 Handling of Withdrawals

All withdrawals regardless of reason (whether the SCOUT PI or the parent removed the child from the study), will be followed-up by a phone call one week after their withdrawal to determine their status and if any medical follow up care was sought (in which case an MCN form will be completed).

The Study Coordinator will collect data from the treating physician by completing the Medical Care Notification (MCN) Form.

Symptomatic subjects that develop a positive urine culture will not be considered withdrawn from the study. They will continue to be seen/contacted for any remaining study visits as part of safety follow-up. These subjects cannot be re-enrolled into this study. For symptomatic subjects with positive urine culture and pyuria during the Day 6 - 10 period the study product will be discontinued. Symptomatic subjects with positive urine culture will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician.

Subjects that withdraw or are withdrawn from the study prior to being evaluable for the primary outcome measure will be replaced.

6.4.5 Termination of Study

The National Institute of Allergy and Infectious Diseases (NIAID), the IRB, and the FDA reserve the right to discontinue the SCOUT Study at any time. Should the SCOUT Study be discontinued prior to completion, the study shall be completed, if medically

appropriate, for subjects who are enrolled in the study. In that event, each enrolled subject will be followed through the period outlined in the study.

7 STUDY INTERVENTION/STUDY PRODUCT

7.1 Study Product Description

The UPENN School of Medicine will provide scientific expertise and will supply the study product and placebo through the Investigational Drug Service (IDS) of the Institute for Translational Medicine and Therapeutics. The study product will be prepared through the IDS for both CHOP and PITT. The CHOP and PITT Investigators and study team staff will be blinded to treatment arm assignment.

For practical purposes, SCOUT will only enroll children whose physicians initiated therapy with one of the four most commonly used antimicrobials for UTI in children across the two participating clinical sites.

These antibiotics are:

- Trimethoprim- sulfamethoxazole (TMP-SMX)
- Cefixime
- Cefdinir[1]
- Cephalexin

In addition, the UPENN IDS will be compounding matching placebo products for each of the three study product antibiotics (those initially prescribed cefdinir are switched to bioequivalent cefixime). These placebos will contain the following ingredients:

- TMP-SMX placebo: SyrSpend-SF ® Cherry, microcrystalline cellulose NF,, FD&C Yellow #6, carboxymethylcellulose NF, xanthan gum NF, citric acid NF, Tween 80®, sucrose NF, cherry syrup NF, FD&C Red #40, FD&C Yellow #6, Sucrose Octaacetate NF and Methylparaben NF.
- Cefixime: Sucrose granular USP, microcrystalline cellulose NF, xanthan gum NF, colloidal silicon dioxide NF, citric acid NF, and strawberry flavor. .
- Cephalexin: Sucrose granular USP, microcrystalline cellulose NF, xanthan gum NF, colloidal silicon dioxide NF, citric acid NF, strawberry flavor, FD&C Red #40, and Allura Dye.

[1] On occasion cefixime may not be available to the prescribing primary care physician. Under such circumstances it is likely that cefdinir will be prescribed as an alternative agent to cefixime. The two antibiotics have therapeutic equivalence. If a child prescribed cefdinir is enrolled, they will be randomized to either five additional days of cefixime or the cefixime placebo.

If the manufacturer of the active study product changes, only a change in color or flavor of the ingredients for the placebo may be acceptable in order to maintain the blind.

7.1.1 Acquisition

The UPenn IDS will purchase study products and place them into blinded treatment kits. Additional blinded treatment kits will be prepared which will include matching placebos that the IDS will manufacture for the study. Penn's IDS will then transfer the treatment kits to the medication storage areas at CHOP and PITT. CHOP and PITT will be responsible for storing study products at their sites. They will oversee the preparation and dispensing of the three antibiotic (TMP-SMX, cefixime, and cephalexin) study product kits (those initially prescribed cefdinir are switched to bioequivalent cefixime) and placebos for CHOP and PITT as well as the maintenance of proper regulatory documentation. In addition, staff at the CHOP and PITT study product area will keep a log of all study products received and dispensed.

7.1.2 Formulation, Packaging, and Labeling

All study products will be dispensed as oral suspensions only.

The formulations as obtained from the commercial manufacturers are as follows:

- Trimethoprim- sulfamethoxazole (TMP-SMX); Oral suspension containing 200 mg of sulfamethoxazole and 40 mg trimethoprim per 5 mL.
- Cefixime (Suprax®): Powder for oral suspension when reconstituted contains 100 mg of cefixime as a trihydrate per 5 mL for smaller children or 200 mg of cefixime as a trihydrate per 5 mL for larger children.
- Cephalexin: One package of oral suspension when reconstituted containing 250mg of anhydrous cephalexin per 5mL for smaller children and two packages of oral suspension containing 250mg of anhydrous cephalexin per 5mL for larger children.

Active cefixime and cephalexin will be transferred in their original commercial form into new containers strictly for blinding/masking purposes. These two medications will remain in un-constituted powder form until immediately before they are given to the subject. A separate smaller, clear TampAlert® jar will contain the pre-measured amount of sterile water that is needed to reconstitute the antibiotic powder. Trimethoprim-sulfamethoxazole does not require reconstitution and will be given to the subject in the

manufacturer's original packaging. For blinding purposes, bottles for the TMP-SMX placebos will be matched to the original's manufacturer's packaging.

Each study product kit will be individually labeled with dosing instructions, recommended storage conditions, the do not use after date, kit number, "Investigational Use", and that the agent should be kept out of reach of children.

7.1.3 Study product Storage

CHOP and PITT will store and oversee the preparation and dispensing of the three antibiotic (TMP-SMX, cefixime, and cephalexin) study product kits (those initially prescribed cefdinir are switched to bioequivalent cefixime) and placebos and the maintenance of proper regulatory documentation.

At both sites, the study products will be stored in locked file cabinets in the study area. Only study personnel have keys for the study cabinet.

All study products will be stored at room temperature at the study site prior to dispensing. After dispensing, parents/caregivers will be instructed to:

- refrigerate the:
  - cefixime suspension or placebo.
  - cephalexin suspension or placebo
- keep at room temperature:
  - TMP-SMX or placebo

7.2 Dosage, Preparation and Administration of Study Intervention/Study product

Description

The Penn IDS will prepare study treatment kits containing sufficient study product for five days (days six -ten) plus a small overage. Because the study products will be dosed based on body weight, the IDS will prepare two concentrations of cefixime so the site-dispensing pharmacist can choose the study product bottle with the correct range to provide the required weight based volume dosage. For cephalexin, one or two packages of study product will be provided depending on the weight of the child. The TMP-SMX packages will accommodate subjects of all weights, so there will only be one size of TMP-SMX packages prepared. The placebos will be prepared in identical containers and formulated to match their active antibiotics in taste, appearance and thickness. The Study Nurse/Nurse Practitioner/Physician will not be unblinded when they reconstitute the study product because the cefixime and cephalexin placebo powder matches the active powder.

Treatment kits will be pre-packaged and randomized to active or placebo in a 1:1 ratio. The identity of each kit, including the antibiotic, size, active/placebo condition, as well as the site to which it has been shipped, will be registered by the IDS into the WesTrax™ system; this system will then randomize an individual kit to a subject, from the supply of kits which are available for use on-site.

All three study products (those initially prescribed cefdinir are switched to bioequivalent cefixime or its matching placebo) and their matching placebos, will be packaged in bottles with child-resistant closures and a tamper-evident seal, which the Investigator, Nurse Practitioner or Study Nurse will break open at the time that the study product is dispensed.

A treatment kit for five days for Days six - ten will contain:

- One bottle of antibiotic or matching placebo;
- A simple instruction sheet for the parent or caregiver, on which the daily dose is clearly marked;
- An oral syringe and a measuring cup for proper administration; and
- For cefixime, cephalexin or their respective placebo, a pre-measured container of sterile water which the pharmacist, physician, or nurse will use for reconstitution immediately prior to dispensing (for TMP-SMX this is not necessary because the commercial product is stable as a suspension already).
- Larger children prescribed cephalexin will be given two kits of cephalexin or matching placebo.
- Larger children prescribed cefixime will receive the higher concentration of cefixime or matching placebo.
- Active TMP-SMX will be dispensed to the subject in the manufacturer's original packaging.

The SCOUT Physician, Study Nurse, or Nurse practitioner will reconstitute the study product prior to giving the study product to the parent/guardian. Active cefixime will be transferred in its original commercial form into new containers strictly for blinding/masking purposes. Two concentrations will be prepared, a 50mL package of 100mg/5mL suspension for smaller children (with 34mL of pre-measured sterile water in the same package) and a 50mL package of 200mg/5mL suspension for larger children (with 34mL of pre-measured sterile water in the same package). The antibiotic is stable at room temperature and can also be refrigerated. For consistency, once mixed together, the label will identify that the product must be refrigerated and used within 14 days.

Active cephalexin will be transferred in its original commercial form into new containers strictly for blinding/masking purposes. A 200mL package of 250mg/5mL suspension (with132mL of pre-measured sterile water in the same package) will be provided to smaller children. Larger children will receive two packages, each with equal volumes of sterile water. The antibiotic is stable for 14 days refrigerated after reconstitution.

Dosing and Administration
Children will complete five days of their originally prescribed antibiotic and then take the study product according to Table 3 for the following five (5) days. Subjects initially prescribed cefdinir will receive cefixime or cefixime placebo.

Table 3 Oral Antibiotic Dosages

Oral Antibiotic Dosage	
Trimethoprim-Sulfamethoxazole* (TMP-SMX)	8 mg/kg/day of Trimethoprim in 2 divided doses, Max 160mg BID Trimethoprim
Cefixime*	8 mg/kg/day in 1 dose, Max 400 mg
Cephalexin	50mg/kg/day in 3 divided doses,

* The placebo for each of the three antibiotics will be dosed by volume (those initially prescribed cefdinir are switched to bioequivalent cefixime) [49, 50].

Route of Administration

All SCOUT Study products will be administered as an oral suspension by measuring cup or oral syringe.

7.3 Modification of Study Intervention/Study product for a Participant

N/A.

7.4 Accountability Procedures for the Study Intervention/Study product(s)

Study treatment kits will be registered by the Penn IDS into the WesTrax™ system at the time they are packed and shipped to the study storage sites. Staff at each study product storage site will all have a log to record the study product bottles received as well as distributed. The sites will also use the WesTrax interactive voice response (IVR) system.

The WesTrax™ system has a supply management menu for the SCOUT Study with the following system menu options: record the actual shipment date, confirm receipt of study product kit, request replacement, and request additional study product kits.

Additionally, the UPENN Investigational Pharmacy will place a two-part label on the outside of each of the study product kits. One part of the label will remain on the outside of the kit. A second label will be affixed to the study product bottle. The Penn IDS pharmacist will be the only person to perform the unmasking if needed.

Westat, the SCOUT subcontractor for site monitoring, will monitor the study sites, storage areas, the pharmacy dispensing logs, as well as the documentation on study product preparation of the study product kits prior to enrollment as well as throughout enrollment to ensure accountability.

7.5 Assessment of Subject Compliance with Study Intervention/Study product

At the Enrollment Visit, the parent(s) or guardian(s) will be given a subject medication diary. The Study Nurse/Nurse Practitioner/Physician will document the study product kit number, the date of the enrollment visit, and the date the study product was started. The Study Nurse/Nurse Practitioner/Physician will also explain the study product dosage instructions to the child's caregiver(s): after completing five days of the originally prescribed antibiotic to then take the study product for five additional days (days six – ten). The Study Nurse/Nurse Practitioner/Physician will also explain how to use the medication diary to report the daily dose, the date, the time the study product was taken and the date that the study product was stopped. The Study Nurse/Nurse Practitioner/Physician will also explain how important it is to adhere to the instructions to complete the first five days of the originally prescribed antibiotic and then on days six – 10 to take the study product. The Study Nurse/Nurse Practitioner/Physician will collect the subject's medication diary and study product bottle at the Test of Cure Post-Treatment Visit on Day 11 - 14.

Dose Adjustments/Modifications/Delays

Subject families will be told that if the child has missed one dose of antibiotic, they should not double the next dose. Instead, they should take the missed dose as prescribed as soon as they remember. At the Enrollment Visit, the Study Nurse/Nurse Practitioner/Physician will provide the child's parent(s)/legal guardian(s) with a study medication diary to record the day, time, and dose taken, missed doses, reason dose was missed (e.g. forgot to take dose, vomiting after taking study product) to help them to help with adherence and compliance.

7.6 Concomitant Medications/Treatments

All concomitant prescription medications taken anytime during study participation will be reported on the case report forms (CRFs). All concomitant medications will be recorded from the Enrollment Visit (Day 2 – 6) through the end of the follow-up phone call on Day 38-44. The concomitant medication form will be completed if the subject's parent(s)/legal guardian(s) mentions that there are any other medications being taken at any point during the study period. The dates of administration, dosage, and reason for use will also be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications taken at any time during study period.

8 STUDY SCHEDULE

Table 4 Study Schedule

Study Procedure	**Day 2-5 (Day 1 = Start of antibiotic treatment)**	**Day 2-6 (Prior to Taking Day 6 prescribed antibiotic)**	**Day 5-6 (only for subjects enrolled on D2-4)**	**Day 11 - 14 (1 – 4 days after completing the study product)**	**Day 24 – 30 (14 – 20 days after completing the study product)**	**Day 38 - 44 (28-34 days after discontinuation of study product)**	**Day 4 – 44 (Throughout SCOUT Study enrollment)**
Study Point of Contact	**Recruitment Call**	**Enrollment Study Visit**	**Follow-up Contact (phone, e-mail, etc.)**	**Test of Cure Study Visit**	**Outcome Assessment Visit**	**Follow-Up Phone Call**	**As Needed if symptomatic**
Recruitment	x						
Request Study Participation		x					
Verbal consent for screening	x						
Written Informed Consent / Assent		x					
Obtain/review Medical history	x	X					
Symptom Questionnaire	x	x		x	x	x	
Randomization		x					
Take Child's Temperature		x		x	x		x
Clinical Evaluation		x		x	x		x
Assess for suprapubic, abdominal, or flank pain or tenderness		x		x	x		x
Stool Sample**		x		x	x		
Concomitant Medication Form		x		x	x	x	x
Medication Diary				x			

Urine Specimen for Urinalysis & Urine Culture				x	x*		x
Collect SAEs				x	x	x	x
Report Intercurrent Illnesses or Symptoms		x		x	x	x	x
Verify afebrile and no worsening of symptoms since enrollment			x				

*If the Study Nurse is not able to collect a urine specimen at the TOC Visit for asymptomatic subjects, then an attempt will be made to collect a urine sample at the Outcome Assessment Visit.

** A stool sample will not be collected at the unscheduled assessment visits.

8.1 Recruitment and Preliminary Screening

Day 1 (one day from start of prescribed antibiotics): Identify Potential Subjects.

The primary care clinician affiliated with one of the two clinical trial centers identifies potential subjects and informs the eligible (symptomatic and urine culture positive UTI) child and their parent(s)/legal guardian(s) about the SCOUT Study, shares the SCOUT Study informational pamphlet and asks permission for the child's family to be contacted by the study staff. An EHR prompt will remind clinicians to speak to the family about the study. If the family is interested in obtaining additional information or enrolling their child, the primary care clinician will share their contact information with the SCOUT Study Coordinator. The EHR prompt will also allow the clinician to alert study staff if a family should not be contacted about the study or has declined participation. Unless the Study Coordinator is instructed by the treating clinician to refrain from contacting the patient's parent/guardian, he/she will contact the eligible child's parent(s)/legal guardian(s) about the SCOUT Study. However, if the study team does receive a report that the parent/guardian has declined to be contacted, no contact will be made by the SCOUT research team.

At CHOP an IRB approved recruitment email blast will be used to aid in reaching potential subjects seen at the outpatient and urgent care network practices in both Pennsylvania and New Jersey. An honest broker will send the emails utilizing a participant recruitment service based solely on the existence of a urine culture order in the potential subjects' medical record. The email contains a letter from the Principal Investigator at CHOP explaining the details of the study and contact information for the primary Study Coordinator. Parents(s) and legal guardian(s) will have the opportunity to reach out to a study specific email address as well if they are interested in participating in the study or determining if their child is eligible.

Day 2 - 5: Preliminary Screening and Recruiting Period

The Study Coordinator will preliminarily screen[2] a referred subject by reviewing their microbiology and hematology lab report to confirm a UTI and review their medical health record for study eligibility (fever and/or urinary symptoms, pyuria, positive urine culture and susceptibility results, and medical history).

If no exclusion criteria are met, the Study Coordinator will attempt to contact the parent(s)/guardian(s) by phone. The Study Coordinator will read an IRB approved telephone script, and obtain verbal consent from the parent(s)/guardian(s) for screening before proceeding with a brief symptom questionnaire to determine whether the child

experienced any UTI symptoms prior to the diagnosis that were not captured in the EHR. Once obtained, the verbal consent will be documented as per local institutional policy. The Study Coordinator will also explain the study protocol and describes the inclusion/exclusion criteria. The Study Coordinator will answer any questions and concerns the parent/guardian may have.

If it appears that a patient may be eligible based on hematology results and medical record review, the Study Coordinator may contact the parent(s)/guardian(s) by phone before urine culture results have been received. The Study Coordinator will call the parent(s)/legal guardian(s) again once culture results are received to inform them of their child's eligibility status. Patients with cultures that do not meet inclusion criteria will be instructed to continue with initially prescribed antibiotic unless otherwise advised by the prescribing clinician.

If the patient has returned a positive culture, and the parent(s)/legal guardian(s) are still interested and believe their child meets the study eligibility criteria, the Study Coordinator will schedule the Enrollment Visit for a time before the child starts the sixth day of the initial antibiotic therapy. The enrollment visit may proceed even if urine culture sensitivities are still pending at the time of the visit. However, if final sensitivities show that the organism is resistant to the initially prescribed antibiotic, the subject is no longer eligible and should be excluded (or discontinued if the child was already enrolled). Staff will instruct the parent(s)/guardian(s) to continue with the initially prescribed antibiotic unless otherwise advised by the prescribing clinician.

If needed, study staff may contact the parent/guardian again just before the Enrollment Visit to confirm the appointment time and location and to ask whether the child is still experiencing UTI symptoms. If the parent/guardian reports that symptoms are still present, the visit will be rescheduled for a later day (if prior to day 6) or the parent will be instructed to continue administration of original antibiotic as instructed by the treating clinician.

8.2 Enrollment Visit (Day 2-6 from start of prescribed antibiotics)

Prior to taking the sixth day of prescribed treatment

The Study Nurse/Nurse Practitioner/Physician will discuss the SCOUT Study, the goal, the study procedures and risks with the parent(s)/legal guardian(s); answer any questions and address any concerns that they have; have the parent(s) or guardian(s) sign the informed consent form and the child sign the assent form (if $\geq$ seven years of age and enrolled at CHOP) if applicable prior to any study procedures being performed. Study Nurses/Nurse Practitioners/Physicians will also administer the eligibility questionnaire; administer a symptom questionnaire to confirm by history clinical

improvement (afebrile within 24 hours and asymptomatic [absence of dysuria in all children; absence of suprapubic, abdominal or flank pain, urinary urgency, frequency, hesitancy, dysuria in children >2 years and absence of poor feeding or vomiting in children >2 months to 2 years]); take the child's temperature; and perform a targeted pain assessment for any suprapubic, abdominal, or flank pain/tenderness. In addition, a baseline stool sample will be collected and concomitant medications will be reviewed.

The written consent and assent documents, acquired from subject's parent(s) or legal guardian(s) and subject (if age seven or older and enrolled at CHOP) before any study procedures are performed and the child is enrolled in the study, will describe potential risks and benefits of study participation as well as the responsibilities of the study participants, parent(s) or legal guardian(s), and study investigators. This assent document will be written in language understandable to the child (age seven or older).

At the Enrollment Visit, after a potential subject has met the study eligibility criteria, including exhibiting clinical improvement (afebrile and asymptomatic), the child will be given a random treatment assignment to either short-course or standard-of-care therapy. Subjects will be randomized based on the responses of the Study Nurse/Nurse Practitioner or Physician to the WesTrax™ interactive voice response (IVR) system. The system will stratify randomization based on whether:

(1) The UTI was originally associated with fever and

(2) The antibiotic prescribed at initiation of therapy.

If the Enrollment Visit occurs at any time prior to completion of the day 5 dosing of prescribed antibiotic treatment, the child's parent(s)/legal guardian(s) will be advised to complete their originally prescribed medication through day five. The subject would start study product on Day 6 and continue through Day 10. For these subjects enrolled prior to Day 5, confirmation from the parents will be obtained on Day 5 or Day 6 (prior to taking the first dose of SCOUT therapy on Day 6) that the subjects remain afebrile and have not had worsening of symptoms. Any volunteer that develops a fever or worsening symptoms prior to initiating study treatment will be considered an entry failure. If the Enrollment Visit occurs on Day 6, the Study Coordinator will clearly explain that the Enrollment Visit can only occur on Day 6 of antibiotic treatment if the child has not yet started the Day 6 dose of the initially prescribed antibiotics. The Study Nurse/Nurse Practitioner/Physician will confirm (at the Enrollment Visit) that the child has not yet started their Day 6 dose of prescribed antibiotics prior to enrolling them through the "Enrollment Visit Case Report Form.'

The Study Nurse/Nurse Practitioner/Physician will review the contents of the study product kit with the child's family, review the need to complete the study product at the dose and frequency indicated and teach them how to complete the medication diary to document adherence.

Parents will be educated at the time of their child's enrollment in the study about the potential sequelae of untreated UTI and the benefits of prompt and adequate treatment. They will be instructed to (1) contact primary care providers and study personnel in the event of intercurrent febrile illness, (2) have their child evaluated within 36 hours by a SCOUT study physician or the child's primary care provider and (3) have a urine sample obtained to evaluate for the presence of UTI. The study nurses, nurse practitioners and the investigators at each site will be available on a 24-hour basis, through a study-dedicated cellular phone.

Stool specimens will be collected either by inoculation of a sterile dacron-tipped swab into stool in a diaper, or by insertion of a swab into the distal rectum (similar to obtaining a rectal temperature) where it will be held in this position for five seconds.

For each subject at CHOP who agrees to participate in the Microbiome pilot study, an additional perirectal swab and anal swab will be collected. The additional swabs will be used to determine the composition of the subjects' microbiome. The anal swab will be collected by brushing the swab back and forth five times against the skin just outside of the rectum. For subjects in diapers, a third swab will be collected from the stool in the diaper if one is available.

Primary care providers will be notified of their patient's participation in the study via telephone, letter, email or electronic health record message.

CHOP Study Visits

Parents of children recruited at CHOP will be given the option of coming to the Wood building at CHOP, meeting the Study Nurse/Nurse Practitioner/Physician at their local primary care practice site, or having a study staff conduct the study visit in their home.

PITT Study Visits

Children recruited at Children's Hospital of Pittsburgh will have the study visit conducted at the Primary Care Center of Children's Hospital of Pittsburgh or at one of the community offices where PITTnet personnel are available.

Subjects will be given appointments at the time of study entry for the Day 11 – 14 Test of Cure Visit and the Day 24 – 30 Outcome Assessment Visit. The Study Nurse/Nurse Practitioner/Physician will explain that during each study visit, the Study Nurse/Nurse Practitioner/Physician will ask a specific series of questions to determine the resolution of UTI related symptoms. These study visits will consist of a verbal clinical assessment (symptom questionnaire) and a targeted pain assessment. For subjects enrolled before Day 5, the Study Nurse/Nurse Practitioner/Physician will explain that verification will be obtained on Day 5 or prior to taking the first dose of SCOUT therapy on Day 6 that the subject remains afebrile and with no worsening of symptoms. Any subject that develops a fever or worsening of symptoms (prior to starting study product) will be withdrawn from

the study and considered an entry failure. These subjects will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician.

Days Six -Ten *(While on the study product: continued antibiotic or placebo)*:
During the five days (days six – ten) that the subject is taking the study product, if the child presents any UTI symptoms (fever or dysuria for all children; absence of suprapubic, abdominal, or flank pain or tenderness; urinary urgency or frequency for children > 2 years of age; poor feeding or vomiting for children ≥ 2 months to 2 years of age) or if the child experiences diarrhea or develops a rash (as a reaction to the antibiotic) the parent or guardian will be instructed to notify the Study Nurse, Nurse Practitioner, Physician or Study Coordinator. They will be seen within 36 hours by one of the SCOUT Study physicians or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician. All symptomatic children will have a urine sample taken for urinalysis and culture. If a visit with SCOUT Study physicians cannot be scheduled, urinalysis and culture results from the PCP visit can be used for study endpoints. If the urinalysis is positive for pyuria and the urine culture is positive, the symptomatic child will stop the study product, and will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician. If the families prefer to see their PCP or pediatrician, the Study Nurse, Nurse Practitioner, Physician and/or Study Coordinator will help to facilitate the follow- up appointment. The Study Coordinator will share all pertinent information related to the study with the physician. Subjects who develop adverse reactions to study product will discontinue the study product.

8.3 Test of Cure (TOC) Post Treatment Visit

Day 11 – 14 *(one – four days after completing the study product)*

Subjects will return for a follow-up visit two – four days after completing the study product. At this follow-up visit, a Study Nurse/Nurse Practitioner/Physician will perform assessments of the subjects. Using a standardized interview form (symptom questionnaire), the subjects and/or their parent(s) or legal guardians will be asked to provide a subjective evaluation of the presence or absence of UTI symptoms and possible side effects of the antibiotic like diarrhea or a rash. Parent(s) or legal guardian(s) will also be asked to report any inter-current illnesses (e.g., upper respiratory infection, pharyngitis, otitis media, sinusitis), the name and duration of concomitant therapy, and potential side effects of study product. They will also be asked if the subject sought medical care for possible recurrence of UTI, the records of the encounter including microbiology culture results will be obtained so the SCOUT Study physician,

nurse practitioner or nurse can follow up with the child's parent(s)/legal guardian(s) and PCP. The Study Nurse/Nurse Practitioner/Physician will collect a second stool sample, the medication diary and the study product bottle. Subjects at CHOP who agree to the Microbiome sub-study will have additional peri-rectal, anal, and stool swabs collected, if applicable. The subject's temperature will also be taken and the child will be assessed for any suprapubic, abdominal, or flank pain or tenderness. In addition, the Nurse will collect a urine sample for urinalysis and culture from all subjects. The Study Nurse/Nurse Practitioner/Physician will use the bag technique on asymptomatic infants and young children (before they are toilet trained) and the clean catch technique for older toilet trained children. For symptomatic infants and young children a urine sample will be obtained using the clean catch technique (or catheterize if unable to capture with clean catch).

If the subject is symptomatic and the urine culture is positive, the child will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician (See Table 5 for details on which scenarios require referrals to non-SCOUT physicians at or after the Test of Cure Visit.). Subjects that are asymptomatic with a positive urine culture (specimen collected via bag) will continue with the study as planned. NOTE: Subjects that are diagnosed with a culture positive UTI prior to the TOC Visit (between Day 6 and Day 11-14 TOC Visit) will have the above listed procedures performed during the Interim Visit for reoccurrence of symptoms excluding the stool specimen collection. Data obtained from this visit will apply towards the primary endpoint. They will not have to return for the TOC visit.

Table 5: SCOUT Study Referrals to outside physicians at TOC or later.

Scenario	UTI Symptomology		Urine culture result		Pyuria present		Follow-up required:	Repeat urine culture result/outcome	
	Symp	Asymp[1]	+	-	Yes	NO			
1	X		X		X		Refer to non-SCOUT physician		
2	X		X			X	Refer to non-SCOUT physician		
3	X			X	X		Follow-up required. Contact subject in 24-48 hrs. Perform repeat urine culture if still symptomatic. If asymptomatic, continue with study as planned	+ Refer to non-SCOUT physician	- Continue with study as planned, monitoring closely
4	X			X		X	Follow-up required. Contact subject in 24-48 hrs - if still symptomatic - perform repeat culture. If asymptomatic, continue with study as planned	+ Refer to non-SCOUT physician	- Continue with study as planned, monitoring closely
5		X	X		X		Asymptomatic Bacteriuria, continue with study as planned		

6		X	X			X	Asymptomatic Bacteriuria, continue with study as planned		
7		X		X	X		Continue with study as planned		
8		X		X		X	Continue with study as planned		

Also, for asymptomatic subjects if a urine sample cannot be obtained at this visit, another attempt to obtain a urine sample will be made at the Day 24-30 Outcome Assessment Visit.

8.4 Outcome Assessment Visit

Day 24 - 30 (14 – 20 days after completing the study product):

The Study Nurse/Nurse Practitioner/Physician will administer the symptom questionnaire, take the child's temperature, and collect a third stool sample. Subjects at CHOP who agree to the Microbiome sub-study will have additional peri-rectal, anal, and stool swabs collected, if applicable. In addition, the child will be assessed for any suprapubic, abdominal, or flank pain or tenderness. The purpose of this visit is to assess for recurrent infections (relapse or reinfection).

If applicable, for asymptomatic subjects for whom no urine sample could be obtained at the TOC visit another attempt will be made to obtain a urine sample at this visit. This sample is taken as a safety measure.

If the subject is symptomatic and the urine culture is positive, the child will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician. If the families prefer to see their PCP or pediatrician, the Study Nurse, Nurse Practitioner, Physician and/or Study Coordinator will help to facilitate the follow up appointment. The Study Coordinator will share all pertinent information related to the study with the physician.

All of the above procedures will be performed for subjects that were categorized as treatment failures or diagnosed as having asymptomatic bacteriuria prior to this visit. These procedures include stool specimen collection.

8.5 Follow-Up Phone Call

Day 38 – 44 (*28-34 days after completing the study product*):

The Study Nurse, Nurse Practitioner, Physician or Study Coordinator will follow up by phone with the subject's parent(s)/legal guardian(s) to administer the symptom questionnaire. The purpose of this call is safety and to evaluate for the presence or absence of a new urinary tract infection.

Using a standardized interview form (symptom questionnaire), the subjects and/or their parent(s) or legal guardians will be asked to provide a subjective evaluation of the presence or absence of UTI symptoms. Parent(s) or legal guardian(s) will also be asked to report any inter-current illnesses (e.g., upper respiratory infection, pharyngitis, otitis

media, sinusitis), the name and duration of concomitant therapy, and potential side effects of study product. If the subject sought medical care for possible recurrence of UTI, the records of the encounter including microbiology culture results will be obtained so the SCOUT Study physician, nurse practitioner or nurse can follow up with the child's parent(s)/legal guardian(s) and PCP. Subjects that were categorized as treatment failures prior to this visit will be asked about the presence or absence of UTI symptoms and the follow-up treatment received.

All children that report symptoms associated with UTI during the phone call will have a visit scheduled to collect a urine sample for urinalysis and culture, and will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician. If the families prefer to see their PCP or pediatrician, the Study Nurse, Nurse Practitioner, Physician and/or Study Coordinator will help to facilitate the follow up appointment if needed. The Study Coordinator will share all pertinent information related to the study with the physician. A SCOUT Study physician, nurse practitioner or nurse will follow up with the PCP/pediatrician on the child's status.

8.6 Early Termination Visit

Subjects that choose to withdraw or are withdrawn by the PI prior to the Day 11-14 TOC visit will be asked to be evaluated for UTI symptoms and possible side effects of the antibiotic like diarrhea or a rash. Parent(s) or legal guardian(s) will also be asked to report any inter-current illnesses (e.g., upper respiratory infection, pharyngitis, otitis media, sinusitis), the name and duration of concomitant therapy, and potential side effects of study product. They will also be asked if the child sought medical care for a possible recurrence of UTI. However, no urine or stool sample will be obtained. If the subject presents with UTI symptoms at the termination visit, the SCOUT PI will inform their PCP/pediatrician and share all pertinent information related to the study and urge the parent(s)/legal guardian(s) to follow-up with their PCP/pediatrician.

Study staff will request that subjects that choose to withdraw or are withdrawn any time after the TOC visit be contacted for the Day 38-44 follow-up phone call for assessment of adverse events.

9 STUDY PROCEDURES/EVALUATIONS

9.1 Clinical Evaluations

Subjects will be seen by a clinician for three study visits:

1. Enrollment Visit (Day 2 - 6)
2. Test of Cure Visit (Day 11 - 14)
3. Outcome Assessment Visit (Day 24 - 30)

Subjects enrolled prior to Day 5 of their prescribed medication will be verified as afebrile and with no worsening of conditions on Day 5 or prior to receiving the first dose of SCOUT therapy on Day 6. In addition, subjects will be contacted by phone at the end of the study period on the Follow-up Phone Call (Day 38 - 44).

Throughout the study, the study nurse, nurse practitioner, MD, PA, DO or DDS listed on the form FDA 1572 may perform and document study assessments and confirm eligibility. Only clinical personnel licensed to perform pain assessments will complete these procedures.

A diagnosis of clinical and microbiological failure as well as asymptomatic bacteriuria will only be made by a MD, PA, DO, DDS or CRNP listed on the form FDA 1572. Clinical evaluations at each visit will be as follows:

Day 2-6 (anytime after initiation of antibiotic treatment but prior to starting the sixth day of study treatment):

To confirm eligibility including clinical improvement (afebrile and asymptomatic) a pain assessment will be performed checking for any suprapubic, abdominal, or flank pain or tenderness. In addition, the temperature will be taken and a base line stool sample will be obtained. If a study RN receives a report of a UTI symptom at a study visit, he/she will inform the parent/guardian that the child should be seen by a clinician licensed to diagnose and treat the UTI. If this occurs at the enrollment visit, the child will be excluded from the study and will be referred back to the PCP who initially treated the child.

Confirmation is obtained by study staff that the subject remains afebrile with no worsening of symptoms on Day 5-6 (prior to taking the first dose of SCOUT therapy on

Day 6) for subjects enrolled prior to Day 5. Any volunteer that develops a fever or worsening of symptoms will be withdrawn from the study and considered an entry failure. These subjects will be treated outside this protocol by a physician not associated with the SCOUT Study per current standard of care or if they prefer the parent(s)/legal guardian(s) can choose to have their child treated by their PCP/pediatrician.

Day 11 -14 Test of Cure Post Treatment Visit (*one – four days after completing the study product)*:

The child will be clinically assessed for the presence of UTI symptoms and a urine specimen will be collected for test of cure. In addition, a pain assessment will be performed checking for any suprapubic, abdominal, or flank pain or tenderness and the child's temperature will be taken and a stool sample will be obtained. If a study RN receives a report of a UTI symptom at the Test of Cure visit, he/she will inform the parent/guardian that the child should be seen by a clinician licensed to diagnose and treat the UTI. The RN will document the symptom on the appropriate case report form and will contact a study physician to arrange follow-up visit.

Day 24 - 30 Outcome Assessment Visit (14-20 days following study product completion): The child will be clinically assessed for the presence of UTI symptoms. In addition, a targeted pain assessment will be performed checking for any suprapubic, abdominal, or flank pain or tenderness and the child's temperature will be taken and a stool sample will be obtained. If a urine specimen could not be collected at the Day 11 – 14 TOC Visit for asymptomatic subjects, another attempt to collect a urine sample at this visit will be made. If a study RN receives a report of a UTI symptom at the Outcome Assessment visit, he/she will inform the parent/guardian that the child should be seen by a clinician licensed to diagnose and treat the UTI. The RN will document the symptom on the appropriate case report form and will contact a study physician to arrange follow-up visit.

Day 38 - 44 Follow-up Phone Call (*28-34 days after completing the study product*): Subjects will be asked regarding presence or absence of UTI symptoms, if they sought medical care for possible recurrence of UTI, and to report any inter-current illnesses.

9.2 Laboratory Evaluations

The Infectious Disease Research Laboratory (IDRL) at Children's Hospital of Pittsburgh (PITT) of the University of Pittsburgh Medical Center will serve as the central microbiology core laboratory for the stool sample analysis for both clinical trial centers.

The CHOP and PITT microbiology and hematology laboratories will analyze the urine culture and urinalysis specimens respectively.

9.2.1 Clinical Laboratory Evaluations

Stool samples

Upon arrival in the IDRL, stool swabs will be initially inoculated onto Maconkey agar. All isolates that grow on Maconkey agar will have identification confirmed using API identification strips. Isolates confirmed to be *E. coli* or *K. pneumoniae* will undergo further evaluation and will be subcultured onto 5% sheep blood agar. Isolates of *E. coli* will undergo screening for the presence of antimicrobial resistance against amoxicillin, amoxicillin- clavulanate, TMP-SMX using Kirby-Bauer Disk diffusion according to published CLSI guidelines (ref). Isolates of *K. pneumoniae* will undergo screening for the presence of antimicrobial resistance against amoxicillin- clavulanate and TMP-SMX using Kirby-Bauer Disk diffusion according to published CLSI guidelines (ref). Isolates of either pathogen found to have a zone of inhibition consistent with them being non-susceptible (either intermediate or resistant) will undergo MIC determination against the relevant antimicrobial agent(s) using the E-test (bioMerieux, Durham, NC).

Screening for third generation cephalosporin resistance for all isolates of *E. coli* and *K. pneumoniae* will be done using selective media: Maconkey agar containing subinhibitory concentrations of ceftazidime. Isolates of *E. coli* or *K. pneumoniae* that have grown on this selective media will be further tested to determine their MIC to ceftazidime using the E-test. In addition to determination of the MIC, isolates of either of these pathogens that grow on the ceftazidime-containing selective media will be screened for the presence of ESBL production using the ESBL strip ceftazidime / ceftazidime + clavulanic acid (AB BioDisk). Isolates of *E. coli* or *K. pneumoniae* that are resistant to any of the four (those initially prescribed cefdinir are switched to bioequivalent cefixime or its matching placebo) antimicrobials (or that demonstrate ESBL production by double disk synergy test) will be frozen at -70 C for future evaluation including determination of the mechanism of resistance and epidemiological relationships.

Each isolate of *E. coli* and *K. pneumoniae* will be defined as demonstrating an MIC that is susceptible, intermediate (where appropriate) or resistant for the specific antibiotic. For TMP-SMX, the breakpoint for susceptible is $\leq$ 2 ug/ml TMP and $\leq$ 38 ug/ml SMX while resistance is $\geq$ 4 ug/ml TMP and $\geq$ 76 ug/ml SMX. For Amoxicillin-Clavulanate, susceptible is $\leq$ 8/4 ug/ml, intermediate is 16/8 ug/ml and resistant is $\geq$ 32/16 ug/ml. Although a similar approach could be offered for ceftazidime resistance in *E. coli*, most experts would consider all ESBL-containing *E. coli* to be resistant to ceftazidime regardless of the MIC. Accordingly, we would argue that this secondary endpoint would be based on the presence or absence of ESBL as determined by the double-disk diffusion test for synergy between clavulanic acid and both ceftazidime and cefotaxime. An increase of > 5 mm in zone diameter for either antimicrobial agent tested in

combination with clavulanic acid compared with its zone when tested alone will be confirmation of the ESBL phenotype.

For each enrolled research subject, the second stool or perirectal swab will also be utilized to perform additional screening for the presence of carbapenem resistant enterobacteriaceae. These second swab specimens will be streaked on to Carbapenem resistant Enterobacteriaceae agar (Hardy's Diagnostic Company). This media selects against growth of Gram positive bacteria and selects for growth of Gram negative bacteria with decreased susceptibility to carbapenems. The carbapenem resistant Enterobacteriaceae plates will be incubated in ambient air at 37ºC for 24 hours. Per the manufacturer's recommendation, Carbapenem resistant Enterobacteriaceae plates with no growth at 24 hours will be considered negative. Colonies that grow at 24 hours are presumed to be Gram negative bacteria and will undergo subsequent formal identification (using API E20 test kits) and susceptibility testing against meropenem, imipemen and ertapenem by E-test in accordance with guidelines from the Clinical Laboratories Standards Institute to determine presence or absence of carbapenem resistance. Rates of colonization by carbapenem-resistant Enterobacteriaceae (overall and for specific pathogens) will be determined.

Urine samples

The site's microbiology and hematology labs will run tests on the urine as per standard CHOP and PITT procedures for a urine culture and urinalysis. These standards include the following differences in procedures:

- Cultures will be checked for growth after
  - CHOP: 20-24 hours.
  - PITT: at least 18 hours.
- Cultures are reported as "No growth" if
  - CHOP: $< 10^2$ CFU/ml
  - PITT: there is no growth
- Cultures will be inoculated with:
  - CHOP: blue (10µl) loops or green (1µl) loops
  - PITT: green (1µl) loops only
- Individual colony counts will be reported for
  - CHOP: up to 3 organisms
  - PITT: up to 2 organisms:
    - If the two organisms are between $10^4 – 10^5$ CFU/ml, ID and sensitivities are performed on each of them
    - If one is $>10^4$ CFU/ml and one is $<10^4$ CFU/ml, ID and sensitivities performed on the former and the latter is only described

Routine Evaluations

When clinically appropriate, routine laboratory measurements will also be performed according to the medical judgment of the investigators.

9.2.2 Special Assays or Procedures

N/A

9.2.3 Specimen Preparation, Handling, and Shipping

9.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Urine

A urine specimen will be collected for urinalysis and culture at the Test of Cure visit. If a urine specimen is not able to be collected for asymptomatic subjects, the Study Nurse/Nurse Practitioner/Physician will attempt to collect a urine specimen at the Outcome Assessment Visit. A urine specimen will also be collected at any time during study enrollment if the child presents with UTI symptoms.

Urine specimens will be collected by various methods depending on the age of the subject and the symptoms, as detailed in the table below.

Table 6: Urine Specimen Collection by Age and Symptom Status

Child Age and Status	Method of Urine Collection
Asymptomatic infants and young children	Bag technique
Asymptomatic toilet trained children	Clean catch technique
Symptomatic infants and young children	Clean catch or catheterize
Symptomatic toilet trained children	Clean catch technique

Urine specimens for local analysis will be delivered to the site's microbiology laboratory within one hour of collection, or kept in a box containing ice cubes, or refrigerated until delivered to the lab.

If an asymptomatic child does not urinate during the allotted TOC Visit time period, the Nurse will request additional time from the parents, but if they are not able to have the study visit continue, then the nurse will attempt to collect a urine specimen at the Outcome Assessment Visit on Day 24 - 30.

Stool samples

A stool specimen to determine antimicrobial resistance will be collected at all three study visits. Stool specimens will be collected either by inoculation of a sterile dacron-tipped swab into stool in a diaper, or by insertion of a swab into the distal rectum (similar to obtaining a rectal temperature) where it will be held in this position for five seconds. The rectal swabs or stool samples for both clinical sites will be tested, stored, and shipped to the Infectious Disease Research Laboratory (IDRL) at Children's Hospital of Pittsburgh (PITT). These samples will be analyzed at a central lab to determine the presence of antimicrobial resistance in isolates of *E. coli* and *K. pneumonia.*

At CHOP, study staff will send collected specimens directly to the IDRL at PITT via UPS. Study staff will be trained according to the International Air Transport Association (IATA) requirements. The specimen and the UPS tracking number will be logged in the database before shipping to the IDRL.

Inoculated swabs will be placed in Cary Blair transport media. All specimens will be refrigerated immediately upon collection and be shipped the same day via overnight mail to the PITT IDRL. If specimens cannot be immediately shipped, they will be placed in a 2-8°C refrigerator and shipped on the next business day. At CHOP, stool specimens will initially be stored at the specimen collection, processing and point of care core lab of the CTRC at CHOP before they are sent to the PITT IDRL. Study staff will send the specimens collected at the outpatient clinics or at subject's homes (away from the CHOP main campus) directly to the IDRL at PITT. Specimens collected from subjects at Children's Hospital of Pittsburgh will be hand-delivered to the IDRL by study personnel.

For each subject at CHOP who agrees to participate in the Microbiome pilot study, an additional perirectal swab and anal swab will be collected. The anal swab will be collected by brushing the swab back and forth five times against the skin just outside of the rectum. For subjects in diapers, a third swab will be collected from the stool in the diaper if one is available.

9.2.3.2 Specimen Shipment

Inoculated stool swabs will be placed in Cary Blair transport media and will be mailed via UPS to the PITT IDRL of the University of Pittsburgh Medical Center, which serves as the central microbiology core laboratory for this project.

Swabs collected for the Microbiome sub-study will be shipped the same day via overnight mail to the Knights Lab at the University of Minnesota. If specimens cannot be immediately shipped, they will be placed in a -20°C freezer and shipped on the next business day.

10 ASSESSMENT OF SAFETY

10.1 Specification of Safety Parameters

Safety will be evaluated by the collection and analysis of data on serious adverse events (SAEs), clinical laboratory tests, pain assessments, and concomitant medications. The investigator is responsible for documenting all SAEs that are observed or reported throughout the study regardless of their relationship to the study products or procedures.

The study products used in this protocol are established generic products labeled for use in the study population for treatment in the indication, UTI. TMP-SMX cefixime, and cephalexin have been used to treat UTIs in children for decades, have established safety records for pediatric use, and have well described side effect profiles. These are the four most commonly prescribed antibiotics at CHOP and PITT for the treatment of UTIs in children.

The subjects will be followed from the Enrollment Visit through the Day 38 – 44 follow-up phone call (four weeks after completing study product therapy). Subjects on the continued antibiotic treatment arm may have a higher likelihood of developing resistant bacteria than subjects on the placebo arm; however, their risk should be no greater than what occurs in standard clinical practice.

For the purposes of this study, adverse events will be collected from the initiation of the study product (Day 6) through the Day 38 - 44 Follow-up Phone Call (four weeks after finishing the study product).

Study subjects will be educated at the time of enrollment in the study about the signs and symptoms of a relapse UTI and the benefits of prompt and adequate treatment as well as the possible side effects of the study product. The study nurses, nurse practitioners and investigators at each site will be available on a 24-hour basis, through a study-dedicated cellular phone. Study subjects will be asked to contact study staff with any symptoms or complaints potentially related to the intervention. In addition, at each study visit, the Study Nurse/Nurse Practitioner/Physician will perform assessments of the subjects. At each of these contacts and visits, parent(s) or guardian(s) will be asked to report any inter-current illnesses or symptoms. If a study RN receives a report of a UTI symptom at any study visit, he/she will inform the parent/guardian that the child should be seen by a clinician licensed to diagnose and treat the UTI. The RN will document the symptom on the appropriate case report form. If the report occurs at the enrollment visit, the child will be referred back to the PCP that treated that child initially. If the symptom report occurs after the child has started SCOUT medication, the RN will contact a study physician to arrange follow-up visit.

Monitoring of the data is an essential part of the research plan for subject safety. Reporting adverse events and unanticipated problems is an essential part of the research plan for safety and compliance.

10.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

At each contact with the subject (at the two follow-up study visits and the day 38 – 44 follow-up phone call), information regarding adverse events will be elicited through appropriate UTI symptom questioning and pain assessment (at the study visits) for any suprapubic, abdominal, or flank pain or tenderness) and will be immediately recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, medication diaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All adverse events that are identified will be recorded on an appropriate case report form (CRF). The start date, the stop date, the severity of each event, and the Investigator's judgment of the AEs relationship to the study product and/or study procedure will also be recorded on the subject's CRF.

The Investigators are responsible for the accurate documentation, investigation and follow-up of all possible study-related adverse events. Clinical site staff will be given appropriate training on AE system for grading the severity of events during the protocol training session.

All SAEs will be documented on an SAE Reporting form. Sites will fax all AERs to CHOP ((267)-426-0380) and PITT ((412)-692-5807) for immediate review and action if necessary. All AERs and Safety Reports will be tracked in the computerized AE Tracking System, and data on every AER will be entered into the AE database.

Clinical site monitors will review medical records and source documentation during on-site monitoring visits to determine if all AEs were appropriately identified, reported and managed.

Monitoring of the data is an essential part of the research plan for subject safety. Reporting AEs and unanticipated problems is an essential part of the research plan for safety and compliance.

10.2.1 Adverse Events

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study

personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, then the following guidelines will be used to quantify intensity.

Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.

Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Life threatening: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. We will document the duration of each episode characterized as intermittent.

Relationship to Study Products, Study Procedures and Initial UTI: The clinician's assessment of an AE's relationship to the study product, study procedures and initial UTI is part of the documentation process, but it is not a factor in determining what is or is not reported in

the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs will have their relationship to study product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines will be used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

10.2.2 Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,

- A life-threatening adverse event*,

- Inpatient hospitalization or prolongation of existing hospitalization,

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

  * Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Recorded on the appropriate SAE CRF
- Followed through resolution by a study clinician
- Reviewed and evaluated by a study clinician

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

10.2.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

No laboratory tests are planned for this study other than urine and stool analysis. Abnormal clinical findings and laboratory test values (if available) will be documented in the source document. A study clinician will review all study lab results to determine clinical significance. Those meeting criteria of SAE will be reported as per Section 10.4.

10.3 Reporting Procedures

10.3.1 Adverse Events

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic. All AEs collected during the study will be documented on the appropriate CRF. CHOP and PITT are responsible for reporting adverse events to their local IRB in accordance with the local IRB requirements. The PI or a co-investigator at each site will determine the proper response per the research protocol—i.e.: changing therapy, initiating new therapy, or having the subject discontinue study product (of days six - ten).

10.3.2 Serious Adverse Events

If in the opinion of a study physician investigator (PI or co-investigator) the event meets the criteria of a SAE the following procedures will occur.

All SAEs will be:

- Recorded on the DMID SAE reporting form and the SAE CRF by study staff.
- Reviewed by PI or co-investigator, who will assess the event's relationship to study product or procedures. All SAEs will be assessed for relationship to the study research procedures. A causal relationship means that the study participation caused (or is reasonably likely to have caused) the event. This usually implies an association in time between a study procedure and the event.
- Followed by PI or co-investigator until satisfactory resolution or until the PI or co-investigator or deems the event to be chronic or the subject to be stable.
- If an SAE has not resolved at the time of the initial report, a follow-up SAE reporting form must be submitted and a follow-up SAE CRF must be completed. SAEs will be followed until resolution or stabilization if the event is expected to remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

All SAEs regardless of relationship will be recorded on the SAE form and sent by fax within 24 hours of site awareness of the event. Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible. This may include all relevant new or reassessed information (e.g. concomitant medication, medical history). Follow-up information will be submitted to the IRB and DMID Pharmacovigilance Group who will provide this to the DMID medical monitor and clinical program manager. All SAEs will be followed until either resolved or stable.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

Serious adverse events will be reported to the independent Data Safety Monitoring Board (DSMB) according to the guidelines and schedule established by that group in the charter. All adverse events and safety data will be reported to the DSMB through regularly scheduled reports.

The Principal Investigator will promptly notify the IRB of all study on-site SAEs and other unanticipated problems related to research using the CHOP Internal SAE reporting form and in accordance with the following timeline. External SAEs that are unexpected and related to the study intervention should be reported as received using the External SAE form (if applicable).

Table 7: IRB Adverse Event Notification

Type of Internal Adverse Event	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within five calendar days
Internal (on-site) SAEs All other SAEs	72 hours	Within five calendar days
Unanticipated Problems Related to Research	72 hours	Within five calendar days
All other Events	N/A	As per local IRB requirement

10.3.3 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data

or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

10.4 Type and Duration of Follow-up of Subjects after Adverse Events

After the initial SAE report, the investigator will follow each subject and provide further information to the DMID medical monitor on the subject's condition. All AEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts. All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE source document and CRF pages will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

All AEs reported in this study will be followed until resolution or until the investigator and the clinical monitor are in agreement that the AE has stabilized and no additional follow-up is required. This requirement indicates that follow-up may be required for some events after the participant discontinues participation from the study. These events will be reported to the IRB annually, as well as the DSMB, according to the frequency defined by the DSMB Charter.

10.5 Halting Rules

Because of the masked nature of the study design, the treatment failure for UTI cannot be monitored in the short-course arm alone. However, we will monitor the failure rate separately in BOTH arms for the first 100 subjects in each arm. To ensure patient safety the following early halting rule will be applied separately to each study arm:

Stop accrual/suspend if at any stage:
3 among the first 16 patients experience a treatment failure
5 among the first 23 patients experience a treatment failure
6 among the first 29 patients experience a treatment failure
7 among the first 36 patients experience a treatment failure
8 among the first 43 patients experience a treatment failure
9 among the first 49 patients experience a treatment failure
10 among the first 56 patients experience a treatment failure

11 among the first 63 patients experience a treatment failure
12 among the first 69 patients experience a treatment failure
13 among the first 76 patients experience a treatment failure
14 among the first 83 patients experience a treatment failure
15 among the first 89 patients experience a treatment failure
16 among the first 96 patients experience a treatment failure
17 among the first 100 patients experience a treatment failure

The halting probabilities (based on sequential Bernoulli outcomes) of the halting rule above are displayed in Table 8 below.

Table 8: **Probability of stopping in terms of true treatment failure rate for UTI and number of patients enrolled on the study**

	Treatment failure rate		
No. of Subjects	5%	15%	20%
15	0.04	0.40	0.60
20	0.04	0.44	0.65
25	0.04	0.46	0.69
30	0.04	0.48	0.72
35	0.04	0.51	0.75
40	0.04	0.52	0.78
50	0.04	0.56	0.82
60	0.04	0.59	0.86
70	0.04	0.61	0.88
80	0.04	0.63	0.90
90	0.04	0.65	0.92
100	0.04	0.67	0.93

Thus, if the true treatment failure rate is 5%, then the probability of terminating accrual at the 25th patient is 4%. Note that this probability converges to 4% and will be expected to remain at this value for the rest of the study. In contrast, if the true treatment failure rate is 15%, the probability of stopping accrual at the 25th patient is 46%.

In addition, any SAE considered related to the study procedures or products will be reported to the DMID Medical Monitor and DSMB members and a safety evaluation will be conducted by electronic review. Upon reviewing the event members of DSMB or/and DMID MM may request an ad hoc meeting to further discuss it.

The ad hoc meeting of the DSMB will be organized to determine if the study may proceed without changes, should be revised, or even terminated. The PI and/or DMID may request ad hoc meetings of the DSMB

The IRB, NIAID, and the FDA as part of their duties to ensure that research subjects are protected; may discontinue the study at any time. Subsequent review of serious, unexpected and related adverse events by the DMID Medical Monitor, DSMB, the ethics review committee or IRB, NIAID, the FDA, and other regulatory authorities may also result in suspension of further trial interventions/administration of study agent at a site. The FDA, other regulatory authorities, and NIAID retain the authority to suspend additional enrollment and study product administration for the entire study as applicable.

If rates of study product-related SAEs are unexpectedly high, the DMID medical monitor will promptly conduct a safety review involving the DSMB to determine if suspension of enrollment is warranted.

10.6 Safety Oversight

Safety oversight will be under the direction of a DSMB which will consist of at least 3 voting members including a biostatistician experienced in statistical methods for clinical trials and a clinician with relevant expertise. The DMSB will operate under the rules of a DMID-approved charter written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. During the course of the study, the DSMB will meet at least annually to assess safety and efficacy data on each arm of the study. The DSMB will review aggregate safety data for increased rate of occurrence of serious suspected adverse reactions. If halting rules are initiated, more frequent meetings may be held. The DSMB will advise DMID of its findings.

Unmasking intentional or unintentional is strongly discouraged and must be approved by DMID. However, if such event(s) occur, the Principal Investigator and co-Investigators will notify the DMID immediately, so that the DSMB can be made aware and assess the potential impact of the unmasking on the overall integrity of the study.

11 CLINICAL MONITORING

11.1 Site Monitoring Plan

Subcontractor, Westat will provide monitoring and safety oversight for the SCOUT Study for all aspects of clinical site and laboratory monitoring, pharmacy auditing, quality control and adverse event reporting. Their responsibilities in this capacity include development of a site monitoring plan; monitoring of sites for protocol adherence, competence of staff, observance of quality assurance procedures; proper storage and security of study product, maintenance of records and subject eligibility; inspection of IRB approval documents; review of subject records for protocol adherence, accuracy, completeness and source document verification; meetings with study staff to review findings, problems, deficiencies and possible solutions; and reporting through CHOP to the DMID Project Officer to summarize key findings, deficiencies and corrective actions.

Westat site monitors will visit the CHOP and PITT research sites to review a selected proportion of the individual subject records, including consent forms, CRFs and supporting source documentation to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. Eligibility will be confirmed for 100% of subjects; and full chart review will be performed for five percent of subjects, unless performance issues indicate that more extensive review is advisable. Regulatory files will also be inspected to ensure that regulatory requirements are being followed. The clinical monitoring plan will be reviewed and approved by the DMID. In addition, DMID will be sent all monitoring reports and that DMID retains the right to assess the quality of monitoring at any time during the study.

Westat site monitors will also visit the CHOP and PITT study product area to review the overall study product management system, including receipt, storage, disposition and accountability of study products.

The site investigators and study teams will make study documents (e.g. consent forms, case report forms) and pertinent hospital and clinical records available for inspection by the local IRB, the site monitors, the FDA, NIAID, the Office of Human Research Protection (OHRP), or the sponsor's designee for confirmation of the study data. Per DMID Source Documentation Standards for DMID clinical studies, v2, March 16, 2004 - if any original records/source documents cannot be made available to monitors for verification of data collected, a "Certified Copy" of the records can be made and kept as part of a separate research record.

12 STATISTICAL CONSIDERATIONS

12.1 Study Objectives

Primary Objective:

To determine if halting antimicrobial therapy in subjects who have exhibited clinical improvement 5 days after starting antibiotic therapy (short course therapy) have the same failure rate (symptomatic UTI) through TOC (visit Day 11-14) as subjects who continue to take antibiotics for an additional 5 days (standard course therapy).

Secondary Objectives:

To determine if short-course therapy compared to standard course therapy results in similar numbers of children experiencing a recurrent infection (relapse and reinfection).

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with asymptomatic bacteriuria.

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with gastrointestinal colonization of antimicrobial resistant *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae*).

To determine if short-course therapy compared to standard course therapy results in similar numbers of children with clinical symptoms that may be related to a UTI.

To determine if short-course therapy compared to standard course therapy results in similar numbers of subjects with positive urine cultures prior to or at the TOC visit.

Sub-Study Objectives:

To determine if E. coli and K. pneumonia recovered from 2 or 3 stool cultures are more likely to be members of disease-associated subgroups within their respective species (E. coli phylogroups B2 and D, K. pneumoniae clusterKpI)

To determine if treatment-susceptible strains recovered from cultures during treatment (culture #1 for 5-day arm; cultures #1 or #2, 10-day arm) are more likely to be members of disease-associated subgroups;

To determine if treatment-resistant strains of either species are more likely than treatment susceptible strains to be recovered from cultures during treatment

To determine the overall prevalence of colonization with carbapenem-resistant Enterobacteriaceae (overall and for specific pathogens) in ambulatory children after completion of treatment for UTI

To compare the overall biodiversity of the gut microbial community, as measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To compare the overall fraction of gut microbiome community represented by gammaproteobacteria measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

To compare the average ecological similarity between microbial communities within and between treatment groups at each follow-up following short-course and standard-course antibiotic exposure.

To describe the breadth of bacteria present in the stool of children receiving therapy for a urinary tract infection. These data will allow for comparison of aerobic bacteria stool composition in these children and present an opportunity to contrast them to similar data available from other pediatric patient populations.

12.2 Sample Size Considerations

The primary aim of this study is to determine if halting antimicrobial therapy in subjects that show clinical improvement 5 days after starting antibiotic therapy (short course therapy) is as effective as continued therapy through day 10 (standard course) as determined by treatment failure rate (symptomatic UTI) through TOC visit Day 11-14 (6-9 days after starting per protocol therapy).

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The treatment failure rate under the standard of care, R_0, is expected to be 5%. This estimate is based on data from previously published studies [43, 47]. The power and sample size calculations are based on the assumption that treatment failure under the short-course therapy, R_{SC}, will range from 5% to 9%.

In the non-inferiority test, on which power and sample size are based, the null hypothesis that short-course therapy is inferior to standard-of-care therapy is tested against the alternative that short-course therapy is non-inferior. That is, H_0: R_{SC}, > R_0, + δ is tested against H_a: R_{SC}, ≤ R_0, + δ, where δ is the "interval of equivalence," the range

in which the treatment failure rates of the two therapies would be considered clinically equivalent. We believe that an interval of equivalence of 5% would be clinically acceptable; a success rate of 95% for the standard-of-care therapy group when compared to 90% for the short-course therapy group. For all power and sample size calculations, the significance level (chance of incorrectly failing to reject the null hypothesis) is $\alpha = 0.05$.

12.3 Planned Interim Analyses

An interim analysis for the primary outcome measure will be performed once half the subjects have enrolled (to get 336 subjects who are evaluable for the primary endpoint). This analysis will test the null hypothesis that the treatment failure rate under short course therapy is equal to (or less than) that of standard therapy against the alternative hypothesis that the failure rate under short-course therapy is greater than using standard-of-care therapy. This test will be one-sided with $\alpha = 0.05$. The endpoint for this analysis will be the treatment failure rate. The statistical test will be a one-sided test of the hypothesis of equal failure rates between the two study arms.

As part of the interim data analyses, conditional power estimates may be made. The conditional power analysis is a re-assessment of the power given the results of the study to date. Large, early differences imply high conditional power; but small differences in the early part of a study may suggest lower power than initially expected.

The methods for the planned statistical interim analysis will be described and justified in the Statistical Analysis Plan (SAP), including the timing of the interim analyses (i.e., when half of the subjects have completed the study), the scope of the interim analysis including a description of which endpoints will be analyzed (i.e., efficacy analysis of the primary endpoint), and the methods or statistical tests that will be used in the analyses. Note that since the interim analysis is for superiority of standard therapy and the final efficacy analysis is for non-inferiority of short-course therapy, there is no impact of the interim analysis on the Type I error of the final efficacy analysis and thus no adjustments to the significance level are required. The SAP will be written prior to the interim analysis.

12.4 Final Analysis Plan

General Design: The data analysis will consist of several parts. First, descriptive and baseline statistics will be generated for outcome measures for the purpose of describing the study population and comparing the two study arms at baseline. Second, the primary and secondary analyses will be conducted. Finally, primary and secondary outcome measures will be compared between various subgroups of the study participants to assess potential interactions with treatment effects. Details will be provided in the

statistical analysis plan (SAP) which will be finalized prior to the interim analysis. A general description of the planned approach is provided below.

Study Endpoints:

The primary outcome measure is:

Comparison of efficacy, based on symptomatic UTI as assessed at the TOC visit (Day 11-14), between short-course and standard-course of antibiotics.

The secondary outcome measures are:

Comparison of number of subjects that have a recurrent infection (includes a relapse UTI or a reinfection) at any time after the TOC visit (Day 11-14), following a short-course versus standard-course of antibiotics.

Comparison of the number of subjects that become colonized with antimicrobial resistant *Escherichia coli* (*E .coli*) and *Klebsiella pneumoniae (K. pneumoniae)* in the gastrointestinal tract as assessed through Day 24-30, following short-course (5-day) versus standard course (10-day) of antibiotics.

Comparison of the number of subjects with asymptomatic bacteriuria following short-course (5-day) versus standard course (10-day) of antibiotics.

Comparison of the number of subjects with clinical symptoms that may be related to a UTI following short-course (5-day) versus standard course (10-day) of antibiotics.

Comparison of the number of subjects with positive urine culture prior to or at the TOC visit following short-course (5-day) versus standard course (10-day) of antibiotics.

The sub-study outcome measures are:

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Presence of E. coli, presence of K. pneumoniae

Sub-types of E. coli (0=phylogroups A or B1, 1=phylogroups B2 or D), sub-types of K. pneumoniae

Presence of treatment-susceptible E. coli, presence of treatment-susceptible K. pneumoniae

Presence of treatment-resistant E. coli, presence of treatment-resistant K. pneumoniae

Carbapenem-resistant Enterobacteriaceae
Presence of carbapenem-resistant Enterobacteriaceae (overall and for specific pathogens).

Microbiome pilot study
Comparison of the overall biodiversity of the gut microbial community, as measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

Comparison of overall fraction of gut microbiome community represented by gammaproteobacteria measured by culture-independent marker gene sequencing at each follow-up visit following short-course and standard-course antibiotic exposure.

Comparison of average ecological similarity between microbial communities within and between treatment groups at each follow-up following short-course and standard-course antibiotic exposure.

Spectrum of Stool Bacteria
Description of the bacteria present on the aerobic stool cultures obtained at each the three study time points.

Descriptive Analysis: Standard descriptive statistics will be used to describe subjects' baseline characteristics at the time of enrollment, as well as study outcome measures overall and within each treatment group. Summary statistics such as means, standard deviations, medians, and ranges will be produced for continuous variables. Frequency counts and percentage will be generated for describing variables that are dichotomous or polytomous in nature. The balance of baseline measures (i.e., at the time of enrollment) between short-course therapy group and standard-of-care therapy group will be compared using two-sample tests, including *t*-tests, the Mann-Whitney test, chi-square tests, and so forth, as appropriate.

Primary Analysis / Efficacy Analysis: The primary analysis will follow Intent-to-Treat (ITT) principles, with subjects analyzed according to randomized treatment assignment. In addition, a "per-protocol" analysis will be performed; the per-protocol analysis would include only subjects who were adherent to their assigned study regimen and completed 80% or more of the prescribed dose. All subjects taking at least one dose of study drug(s) who have been evaluated for treatment success at TOC visit or have failed treatment prior to TOC will be included in the ITT analysis (see the table below). The primary analysis will be a non-inferiority test comparing the number of symptomatic UTIs at the TOC visit between two treatment arms along with the 5% equivalence interval. The confidence interval of the difference in symptomatic UTI rates between the short-course and standard-of-care therapies will be calculated to evaluate whether this difference is less than or equal to the equivalence interval. This will be a one-sided test with $\alpha = 0.05$. Subjects with asymptomatic bacteriuria or who are asymptomatic with a

positive culture including pyuria will NOT be considered treatment failures for the primary outcome measure.

Table 9: SCOUT Study product Dosage Adherence

Antibiotic Drug	Antibiotic Dosage	80% of Expected Doses
Trimethoprim-Sulfamethoxazole (TMP-SMX)	8 mg/kg/day of Trimethoprim in 2 divided doses, Max 160mg BID Trimethoprim	Eight doses
Amoxicillin-Clavulanate*	45 mg/kg/day in 2 divided doses, Max 875 mg Q12H	Eight doses
Cefixime	8 mg/kg/day in 1 dose, Max 400 mg	Four doses
Cephalexin	50mg/kg/day in 3 divided doses	Twelve Doses

*Amoxicillin-clavulanate is no longer included as a study antibiotic, but subjects randomized to this treatment prior to its removal must have taken at least eight doses to be included in the per-protocol analysis.

Secondary Analysis:

The secondary outcome measures of this trial are provided above. A difference between the two arms with regards to asymptomatic bacteriuria, presence of clinical symptoms, and negative urine culture is not expected. No difference will mean that shorter courses of antibiotics for treatment are not inferior to long courses in eradicating bacteria from the urinary tract as well as impacting clinical symptoms. This analysis will be a conventional test for efficacy and will consist of a straightforward two-sample test for a difference between proportions, using a two-sided chi-square test at significance level of .05.

Sub-study Analysis:

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As previously noted, patient stool samples will be collected on 3 occasions. For primary analysis, cross-sectional analysis will be performed, i.e. looking at samples from one occasion at a time.

For each occasion, the following proportions will be summarized:

1) Among all patients, proportion of patients with *E. coli* detected, proportion of patients with *K. pneumoniae* detected, and same proportions within each treatment arm.
2) Among patients with *E. coli* detected, proportions of sub-type 0 or sub-type 1; among patients with *K.pneumoniae* detected, proportions of the two sub-types, and same proportions within each treatment arm.
3) Among patients with *E. coli* detected, proportions of treatment-susceptible *E. coli*; among patients with *K. pneumoniae* detected, proportion of treatment-susceptible *K. pneumoniae*; and same proportions for different treatment arms.
4) Among patients with *E. coli* detected AND treatment-susceptible, proportions of sub-type 1; among patients with *K. pneumoniae* detected AND treatment-susceptible, proportions of sub-type 1; and same proportions for different treatment arms
5) Among patients with *E. coli* detected proportion of treatment-resistant *E. coli*; among patients with *K.pneumoniae* detected, proportion of treatment-resistant *K. pneumoniae*; and same proportions within different treatment arms.

After obtaining the proportions listed in (2) above, a binomial test will be used to determine whether the proportion of *E. coli* sub-type 1(phylogroups B2 and D) is significantly larger than 50%. A chi- square test will also be used to test the differences in proportions of sub-type 1 across treatment arms. In addition, a multivariable logistic regression on presence of sub-type 1 strains will be conducted. The same approach will be used for K. *pneumoniae* sub-type 1.

For culture #1 (5-day treatment arm), or culture #1 or #2 (10-day treatment arm), the proportions of sub-type 1 will be tested using a binomial test, then a logistic regression will be used to examine the association between presence of subtype 1 strains and treatment arms.

A chi-square test will be used to examine the difference in proportions estimated in (3) and (5) above. Logistic regression models will also be used for analysis.

Microbiome pilot study

We will preprocess all marker-gene microbiome sequencing data using the Quantitative Insights Into Microbial Ecology (QIIME) software package [PMID: 20383131]. This will include performing sequence quality filtering and demultiplexing of reads to separate sequences by sample. For taxonomic marker gene and RNA-Seq sequences, we will stitch overlapping paired-end reads together after demultiplexing using PANDAseq [PMID: 22333067]. We will then map marker gene reads to 97% operational taxonomic units (OTUs) using the Greengenes [PMID: 22134646] reference database and the USEARCH [PMID: 20709691] reference mapping software wrapped by QIIME. We will measure within-sample biodiversity (alpha diversity) based on these OTUs using whole-tree phylogenetic diversity56 and the Greengenes reference phylogeny. To quantify

differences in microbial carriage and composition between and within subjects over time, we will calculate the UniFrac distance [PMID: 20827291] between all pairs of samples. UniFrac is a measure of the fraction of the evolutionary tree relating all observed taxa that is not overlapping between two samples. To visualize these within- and between-subject differences (beta diversity) and to obtain principal components for subsequent statistical testing, we will perform dimensionality reduction using principal coordinates analysis. To enable tests for shifts in the relative abundance of both broad and specific bacterial taxa with respect to the host factors mentioned above, we will collapse the reference-based OTUs according to taxonomy at the genus and phylum levels using QIIME. This will produce culture-independent fractional observation counts of bacterial taxa in all samples.

We will test for overall differences in microbiome composition between short-course and standard-course antibiotics. To test for associations between individual bacterial taxa and these groups, we will perform non-parametric Mann-Whitney U tests. We will perform a similar test for association of microbiome biodiversity with treatment and between-sample diversity as well as gammaproteobacteria relative abundance.. In all cases the significance of association will be determined after correcting for multiple comparisons using false discovery rate.

Subpopulation Analyses: Treatment effects for the primary and secondary outcome measures will be estimated as differences between proportions and compared for subpopulations defined by age groups, febrile or non-febrile UTI (at original UTI diagnosis), and type of originally prescribed antibiotic. First, proportions will be tabulated as two-sample comparisons and chi-square tests for treatment differences in the outcomes within subgroups will be calculated. Factors where significant differences are observed will be further assessed using logistic regression analysis, assuming that the logistic model fits the data. Goodness of fit test will be reported for model checking. Treatment-by-subpopulation interactions will be tested using likelihood ratio tests. The power of these comparisons is expected to be low for both primary and secondary endpoints. The study is only likely to detect marked differences in treatment effects in these subpopulations.

Safety Analysis: Safety in this study is related to efficacy. Safety will be evaluated by the collection and analysis of data on SAEs, clinical laboratory tests, pain assessments, and concomitant medications. The investigator is responsible for reporting all SAEs that are observed or reported up, regardless of their relationship to study product. Serious adverse events and abnormal laboratory (if available) results will be compared by treatment arm, with statistical tests for differences in proportion of subjects with serious adverse events between study arms.

Missing Data: Several procedures will be used to conduct data analysis when data for either outcomes or covariates are missing. The first step will be to assess the extent and pattern of missing data. If data are missing for only a few cases, then data analysis will be conducted only on study participants with complete data. However, when such a strategy would result in loss of data from a substantial proportion of participants or would lead to biased or inaccurate results, then some form of imputation will be performed. The form of imputation used will depend on the nature of the data that are missing.

Attrition and Loss to Follow-up: It is expected that loss to follow-up will be minimal in this study. Because the study is of short duration, we are hopeful that we will able to follow virtually all subjects to study completion. However, some study participants may withdraw consent or may not return for the follow-up visits to determine the primary endpoint. Other participants may not have the endpoint determined due to problems with specimens or laboratory processing. We anticipate that these factors may reduce the sample size by as much as 10%. Thus the sample was increased by approximately 11% to offset this sample size reduction. Note that study participants who disrupt or discontinue study treatment will continue with follow-up and have the study endpoints assessed, and will be included in the ITT analysis.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Subject information will only be entered into the database once the informed consent form / assent form (if applicable) has been signed and the child is formally enrolled. Preliminarily screened subject information will be kept in an on-site password protected locked database.

Study data will be collected on case report forms (CRF) designed for the study. The Principal Investigator is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of a piece of data) should support the data collected on the case report form, and in the case of CRFs, they will be signed and dated by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Data for CRFs will be collected during subject visits, phone calls with subjects and health care providers, medication diaries and abstracted from the medical record. The CRF forms will also include the lab results from the subject's initial urine analysis, the test of cure urine sample, and their three stool samples collected at each of the three study visits.

If any original records/source documents cannot be made available to monitor for verification of data collected, a "Certified Copy" of the records can be made and kept as part of a separate research record.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Following a DMID-approved Quality Management Plan approval, the CHOP and PITT sites will be responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Westat, the SCOUT site monitor subcontract, will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to CHOP and PITT for their review. A summary of the site monitoring reports will be included in the monthly safety reports that are submitted to DMID.

The CHOP-BDMC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Ethical Standard

The investigators will ensure that this study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The investigator's Institution will hold a current Federal Wide Assurance (FWA) issued by OHRP for federally funded research.

15.2 Institutional Review Board

A copy of the protocol, informed consent forms, other information to be completed by participants, such as survey instruments or questionnaires, and any recruitment materials, symptom and inclusion/exclusion criteria eligibility script, will be submitted to the IRB for review and written approval.

The investigator must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

In addition, CHOP and PITT will request an IRB waiver to review the urine lab results and medical health record to preliminarily screen a prospective subject for study eligibility prior to consent.

The investigator will notify the IRB of serious adverse events, protocol violations, and unanticipated problems.

Changes to the protocol must be submitted to NIAID for review and approved by the IRB prior to being instituted. A parent or legal guardian of the subject or where applicable the child (age seven or older) must sign written informed consent before the subject undergoes any study procedures. The IRB registration will be submitted to DMID.

15.3 Informed Consent Process

Physicians at participating clinical recruiting sites will identify subjects with UTIs, inform the parent(s)/guardian(s) of the study, share the SCOUT Study informational pamphlet,

and request their permission to be contacted by a study team member if they are interested in participating.

The children's families will be given a SCOUT information pamphlet when their physician first tells them about the study when they initially present with UTI symptoms; the Study Coordinator will tell them more about the study and answer any questions that they might have when he/she calls to screen. If the Study Coordinator obtains verbal consent for screening from the parent(s)/guardian(s), the Study Coordinator will administer a brief symptom questionnaire. If interested, an enrollment visit will be scheduled. At the enrollment visit subjects will be given comprehensive information with the informed consent form and the Study Nurse/Nurse Practitioner/Physician will go over the study procedures with them and answer any questions that they might have before they sign the consent form. Ample opportunity will be made for the Study Nurse/Nurse Practitioner/Physician and the subject and their parent(s)/legal guardian(s) to share information and ask questions. Informed consent is an ongoing, interactive process that is initiated when the discussion regarding participation on the study begins and continues throughout study participation. The Study Nurse/Nurse Practitioner/Physician will discuss the study's purpose, procedures, timeframe, risks, potential benefits, and the rights of study participants with the child and their parent/legal guardian, to help the subject and their parent(s)/legal guardian(s) make educated decisions about whether to begin or continue participation in the trial. Informed consent will be obtained for each study subject prior to the conduct of study procedures. The SCOUT Study Nurses/Nurse Practitioners/Physicians will be well versed on the study protocol as well as on UTIs and will be able to thoroughly explain the study to the children's families as well as answer any questions or concerns that they might have.

The written consent document, signed by the child' parent(s) or legal guardian(s) will describe potential risks and benefits of study participation as well as the responsibilities of the study participants, parents or legal guardians, and investigators, as well as give the parents/guardians an opportunity to consent or decline for the future use of stool samples collected throughout the study. This consent document is written in language understandable to the adult providing consent as the child's responsible representative. The SCOUT Study will require the consent of only one parent or legal guardian to enroll the child in the study (45 CFR §46.408). In addition, age-appropriate assent will be obtained from those children seven years or older. The original signed informed consent form and assent (if applicable) will be retained in the medical chart and a copy will be given to the subject.

Participation in the SCOUT Study is voluntary. Subjects may withdraw consent at any time throughout the course of the study. The acquisition of informed consent will be

documented in the participant's medical records, as required by 21 CFR 312.62. . The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.3.1 Informed Consent/Assent Process (in Case of a Minor)

For this study, all of our subjects will be under the age of 18 years old, therefore their parent(s) or legal guardian(s) will be asked to sign the informed consent form. Written consent will be obtained from the subject after all questions and discussions have been completed. The National Commission for Protection of Human Subjects of Biomedical and Behavioral Research established age seven as a reasonable minimum age for involving children in some kind of assent process. Therefore, age-appropriate assent will be obtained from those children seven years or older.

The study does not involve any other special class of subjects.

15.4 Exclusion of Women, Minorities, and Children (Special Populations)

There are no exclusions.

15.5 Payment to Subjects/Families

All subjects (CHOP and PITT) will be compensated for their time and expenses at $25 per study visit for a total of $75 for the three study visits.

15.6 Subject Confidentiality

CHOP and PITT will institute strict procedures to maintain subject confidentiality. No personal information, such as names, contact information, social security numbers, etc. will be stored in the database managed by the CHOP-Westat BDMC. Subject confidentiality will be maintained by the investigators and the study teams, and by all administrators who are part of the study. Confidentiality will be maintained according to ICH E6; 4.8.10, part O "Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the study are published, the subject's identity will remain confidential."

Information such as dates of birth and dates of events will be stored in the database by SCOUT Study ID number. Physical files linking study ID numbers to personal information will reside in locked files in the office of the principal investigator or the Study

Coordinator at the clinical site where the subject is enrolled. Any publications or presentations resulting from this work will not identify study subjects by name, but will only present aggregate data.

Subjects will not be identified in any published reports of this study. All records will be kept confidential to the extent provided by federal, state and local law. In addition, the investigator shall inform participants that study monitors and other representatives of NIAID may inspect documents and records required to be maintained by the investigator. The investigator will inform the participants that NIAID representatives are bound by agreement and the law to maintain subject privacy and confidentiality. All laboratory specimens, evaluation forms, reports, and other records that leave the collection site will be identified only by a coded number/de-identified in order to maintain participant confidentiality. Personally identifiable information in electronic data management systems will be protected compliant with regulatory requirements and federal computing security requirements to preserve privacy and confidentiality. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, and OHRP.

Both CHOP and PITT have facilities available and procedures in place to ensure the secure storage of subject records in compliance with HIPAA regulations. All physical files linking study records to personal information will reside in locked filing cabinets in the offices of the investigative team members. Access to these locked storage areas will be strictly limited to study investigators. Computers used for storage of study data will be password protected. Subject identifiers will be removed before entering information into the research database.

Seattle Children's Hospital and the University of Minnesota, the institutions conducting the sub-studies, will receive no identifiable patient information.

15.7 Future Use of Stored Specimens

All subject(s) or parent(s)/legal guardian(s) will be asked to sign the informed consent form and specify whether or not they agree to allow their leftover stool samples to be used for secondary analyses. The stool samples may be shared with other investigators at other institutions. The focus is on obtaining permission to analyze archived samples in ways not planned at the time that the initial informed consent was obtained.

Those subjects that do not allow for future use of their stool samples will have their samples destroyed at the end of the study. Subjects who do not agree will indicate this on the informed consent and will have no further involvement in this study. Declining

consent does not in any way jeopardize participation in any other current or future clinical trial.

All children enrolled in the SCOUT Study will have a urine specimen collected after they have completed their study product at their Test of Cure Study Visit (or for asymptomatic subjects at the Outcome Assessment Visit if unable to collect it at the TOC Visit). These urine samples will only be used to determine if their UTI was adequately treated and will not be kept for additional studies. Genetic testing is not planned; however there may be future bacterial genomic testing on the subject's stool sample.

16 DATA HANDLING AND RECORD KEEPING

16.1 Data Management Responsibilities

The CHOP Biostatistics and Data Management Core (BDMC) facility will provide analytical, data management and information technology support.

The objectivity and independence in carrying out statistical support and data management services is ensured through the creation and implementation of the Statistical Analysis Plan (SAP) and Data Management Plan (DMP). The SAP is a written document describing the analyses to be performed for this study. Before any analyses (including interim analyses) begin, the Protocol Biostatistician will develop the SAP in conjunction with other applicable project staff, following the BDMC Standard Operating Procedure (SOP). The SAP addresses statistical analysis considerations such as: justification for the sample size; populations considered for the analysis of efficacy and safety; definitions of eligibility and evaluability, data handling and statistical methods, specifications for how to handle missing data, planned reasons for excluding subjects for whom data are available from analysis, summary of method of analyses, including statistical tests for each of the outcome measures outlined in the protocol, planned analyses of subgroups and project center effects; and justification for adjustment or lack of adjustment to the significance level for multiple comparisons.

The BDMC data management team, led by a Clinical Data Manager (CDM), will develop a Data Management Plan (DMP) that provides information on study objectives; the scope of work; responsibilities; applicable BDMC SOPs; scheduled study visits and required CRFs; data handling conventions; and communication and coordination plans with NIAID/DMID, Principal Investigators (PIs), sites, and internal study staff. The DMP serves as a reference guide to the operational procedures governing the life-cycle of data management activities for the study. This comprehensive plan encompasses all data management phases and facets of the study to ensure high standards of data quality. Having these data management policies and procedures documented prior to the start of data collection, updated as necessary during the study, and monitored for compliance throughout the study, ensures objectivity and independence while carrying out data management services.

In addition, the BDMC data management team will assist with the design of case report forms (CRFs). The BDMC CRF development team will follow BDMC SOPs to design CRFs electronically that are user-friendly for the sites and meet the specific needs of the trial as defined in the protocol. These SOPs are Data Management Case Report Form Review and Developing Case Report Forms. CHOP will work with NIAID/DMID and the

PIs to develop a first draft of the CRFs. They will develop CRF completion guidelines, and provide training to site coordinators on how to complete CRFs. The BDMC data management team will set up data entry screens, program edit checks, perform functional testing, maintain databases, and provide user support. The CHOP-Westat BDMC training team will provide training to data entry staff from participating sites before they begin data entry in OC-Remote Data Capture (RDC) application. They will successfully complete this training before receiving access to the RDC production database. The BDMC data management team will also provide study-specific training.

16.2 Data Capture Methods

Data for all subjects enrolled in this study will be obtained from thirteen sources: 1) the clinical microbiology laboratory; 2) initial subject screening phone call; 3) the Enrollment Visit; 4) Test of Cure Visit on Day 11 – 14; 5) Outcome Assessment Visit Day 24 - 30; 6) Follow-up Call on day 38 - 44; 7) the Pittsburgh Infectious Disease Research Laboratory (IDRL); 8) the subject medical record; 9) urinalysis; 10) urine culture; 11) three stool specimens; 12) medication diary; and 13) medication usage data (medication diary). Since subjects will be recruited from three sources (primary care sites, in-patient units, and emergency department), the medical record encompasses all three sources of data. Data elements provided by each source are shown in the table below. In case of discrepancies in data from different sources, the data source considered primary will take precedent.

The sub-study at Seattle Children's Hospital will analyze isolates from stool samples already collected as part of the SCOUT study. No additional data sources will be used for the sub-study.

The Microbiome pilot study will analyze additional perirectal swabs, anal swabs, and stool specimens collected from subjects who agree to participate. Additionally, subjects agreeing to participate will be asked to complete a questionnaire providing data about feeding (breast milk, formula, timing of introduction of solid foods); tonsillectomy history; most recent antibiotic prescriptions (name, dose, and duration); and number of antibiotic courses in life.

The Spectrum of Stool Bacteria sub-study will collect data on organisms that are already identified as part of the processing of the stool collected for the SCOUT study. No additional data sources will be used for the sub-study.

Table 10: SCOUT Study Data Collection

Data	Initial phone call	Enrollment Study Visit	TOC Study Visit	Outcome Assessment Visit	Follow-up Phone Call	As Needed	Pitt IDRL	Medical Record	AE Form
Preliminary Study Eligibility	**P**								
Confirmatory Study Eligibility		**P**							
Demographics		**P**						**S**	
Medical History	**P**	**P**						**S**	
Symptom Assessment	**P**	**P**	**P**	**P**	**P**	**P**			**P**
Clinical Evaluation		**P**	**P**	**P**		**P**		**S**	
Medication History		**P**						**S**	
Concomitant Medications		**P**	**P**	**P**	**P**	**P**		**S**	**S**
AEs and SAEs			**P**	**P**	**P**	**P**			**P**
Stool Specimen		**P**	**P**	**P**			**P**		
Urine Culture and Urinalysis			**P**			**P**		**S**	
Study product Usage			**P**						
Compliance			**P**						

P-Primary source of data; **S**-Secondary source of data

The CHOP- Westat's BDMC Technical Training Team will use Oracle Clinical (OC) Remote Data Capture (RDC) as the primary data collection and management system for this trial. This system is fully validated and compliant with all federal guidelines. The BDMC database development team will develop the OC-RDC database based on the defined data elements of the CRFs following BDMC SOP, Systems Development Life Cycle. The database developers will maintain and update the database to reflect and track updates to the CRF modules; all of the CRF updates must be approved by the NIAID Division of Microbiology and Infectious Diseases (DMID) before the BDMC begins the database and data entry screen modification. Additional information on the security-relevant features of the application will be added when the system is implemented in support of the proposed research study. The BDMC data management team will develop data entry guidelines and CHOP and PITT site staff will perform web-based, on-site data entry using the RDC application.

Study site staff will perform web-based, on-site data entry using RDC. Users are granted access at the site level so that they are allowed to see only their own data. One of the most valuable features of RDC is its provision of immediate feedback as data are entered. Univariate checks such as missing data and range checks are executed upon data entry and provide an opportunity to correct an error or to create a discrepancy and proceed with data entry. Upon completion of data entry and saving a form, multivariate checks (such as cross form checks) are automatically executed. In addition, edit checks can be executed in batch mode by the user as needed. Users can also add manual discrepancies to a field or sections of a CRF as needed. In order to maximize accuracy of the data entered, BDMC will perform a manual review of data and will utilize the approval and verification features in RDC for quality control purposes at the sites.

The CHOP-Westat BDMC Technical Training Team will provide a web-based training as an option for generic RDC training. In-person, instructor-led training is also available upon request. The generic training will focus on teaching the basic concepts and principles of the RDC application as they apply to the user's role (e.g., entering data). To ensure that users have an opportunity to demonstrate competency in performing RDC tasks before accessing the actual production database, users will be assessed on two levels. The first level will measure the user's comprehension of basic RDC concepts and principles using true/false and multiple choice questions. The second level will allow the user to apply these concepts in a "real world" environment with a practicum training case using electronic CRFs (eCRFs) similar to those used for the protocol. Using the RDC training database, which simulates the production environment, the user will perform tasks on the eCRFs just as he or she would when in the production database. While the training case does not contain exact duplicates of the protocol eCRFs, and therefore does not substitute for protocol or project-specific training, it does give users an

excellent opportunity to transfer and apply the common concepts and principles learned in training to eCRFs resembling those on the protocol. Instructors will be available via email and by appointment during normal business hours to assist users with any questions or concerns that may arise. Users must successfully pass both levels of assessment to receive a certificate of qualification and RDC production access. Users who are not successful will participate in a one-on-one remediation session with an instructor before retaking the assessment.

Additionally, protocol specific RDC training will be provided via web-cast by the data management team. The data entry guidelines document is used as the basis for this training; instructors review the guidelines document with participants and demonstrate the keying of data into RDC by using actual eCRFs in a test database.

The BDMC will use the validated Thesaurus Management System (TMS) module within OC to automatically code all direct matches of the CRF term with the preferred term from the dictionary. The TMS system facilitates the consistent use of dictionaries. The Medical Coder will manually code non-matched terms if the preferred term is obvious. Non-matches that cannot be coded by the Medical Coder are forwarded to the supervising coder for review. Since the study subjects will be otherwise healthy normal children, it is difficult to know in advance how many events or medications will need to be coded, but it is expected to be a relatively small number.

16.3 Types of Data

Data for all subjects enrolled in this study will be obtained from thirteen sources: 1) the clinical microbiology laboratory; 2) initial subject screening phone call; 3) the Enrollment study visit and, for subjects enrolled prior to Day 5, phone call verification for no fever or worsening of symptoms; 4) Test of Cure Visit on Day 11 – 14; 5) Outcome Assessment Visit Day 24 - 30; 6) Follow-up Phone Call on Day 38 - 44; 7) the Pittsburgh Infectious Disease Research Laboratory (IDRL); 8) the subject medical record; 9) urinalysis; 10) urine culture; 11) three stool specimens ; and 12) medication diary. Study data will be collected on case report forms (CRF) designed for the study in an electronic data system. Data for this study will include safety, clinical laboratory, and outcome measures.

16.4 Timing/Reports

The CHOP Biostatistics and Data Management Core (BDMC) has developed a wide variety of status reports to monitor study progress. Study status of data management activities is provided on a frequent basis via a project status report. The report contains the number of CRF pages received, pages entered, pages reviewed, outstanding query

resolutions, and total number of subjects per site. These status reports will be made available to NIAID/DMID on a monthly basis. There are other possible status variables that NIAID/DMID might wish to monitor, and the BDMC will work with NIAID/DMID to define these reports.

The Westat Automated Report Portal (WARP) is a web-based service designed to provide users with an online reporting system. WARP delivers dynamic reports driven by project databases, as well as static documents such as PDF and MS-Word files. Dynamic reports can be programmed and run on-demand. Users simply enter pre-selected report parameters through the WARP user interface and receive dynamic output through their browser. Multiple output formats are available, such as HTML and PDF, and users can extract and download pre-programmed data via Excel files for additional processing.

Once half the subjects have completed the study, an interim analysis for the primary outcome measure will be performed.

At the conclusion of the study, a CD (or other media, if requested) will be sent to NIAID/DMID containing the following:

- Clinical datasets in SAS;
- SAS formats and catalogs; and
- Summary of transmitted datasets.

16.5 Study Records Retention

Study data, case report forms, and regulatory documents will be kept at the sites throughout the duration of the study. At the conclusion of the study, the records will be retained at archival storage places in accordance with the applicable FDA regulations (21 CFR 312.62) as paraphrased below:

1) For at least two years following the date on which the study product is approved by the FDA for marketing for the purposes specified in the clinical investigation; or

2) For at least two years following the date on which the entire clinical investigation (not just the investigator's part in it) is terminated or discontinued. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

16.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice, or protocol specific Manual of Procedures. The noncompliance may be on the part of the subject, the investigator, or the study site staff. If study staff is unable to contact a subject requiring the day 5/6 contact before the subject starts the study product on day 6, it will be categorized as a protocol deviation.

All protocol deviations will be reported to DMID within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations from the Protocol must be addressed in the Subject Source Documentation. The documentation should include the reasons for the deviation and all attempts to prevent or correct them. Protocol deviation reports will be submitted as required by site IRBs.

17 PUBLICATION POLICY

Following completion of the study, the investigator will publish the results of this research in a scientific journal. The DMID will review the publication prior to submission to a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This protocol will be registered on ClinicalTrials.gov by the Principal Investigator, as sponsored by the National Library of Medicine, to meet publication requirements by the ICMJE. The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome.

Authorship of publications developed from this study will be determined by Dr. Zaoutis in collaboration with Dr. Hoberman. Prior to submission for publication, articles will be submitted to DMID for review.

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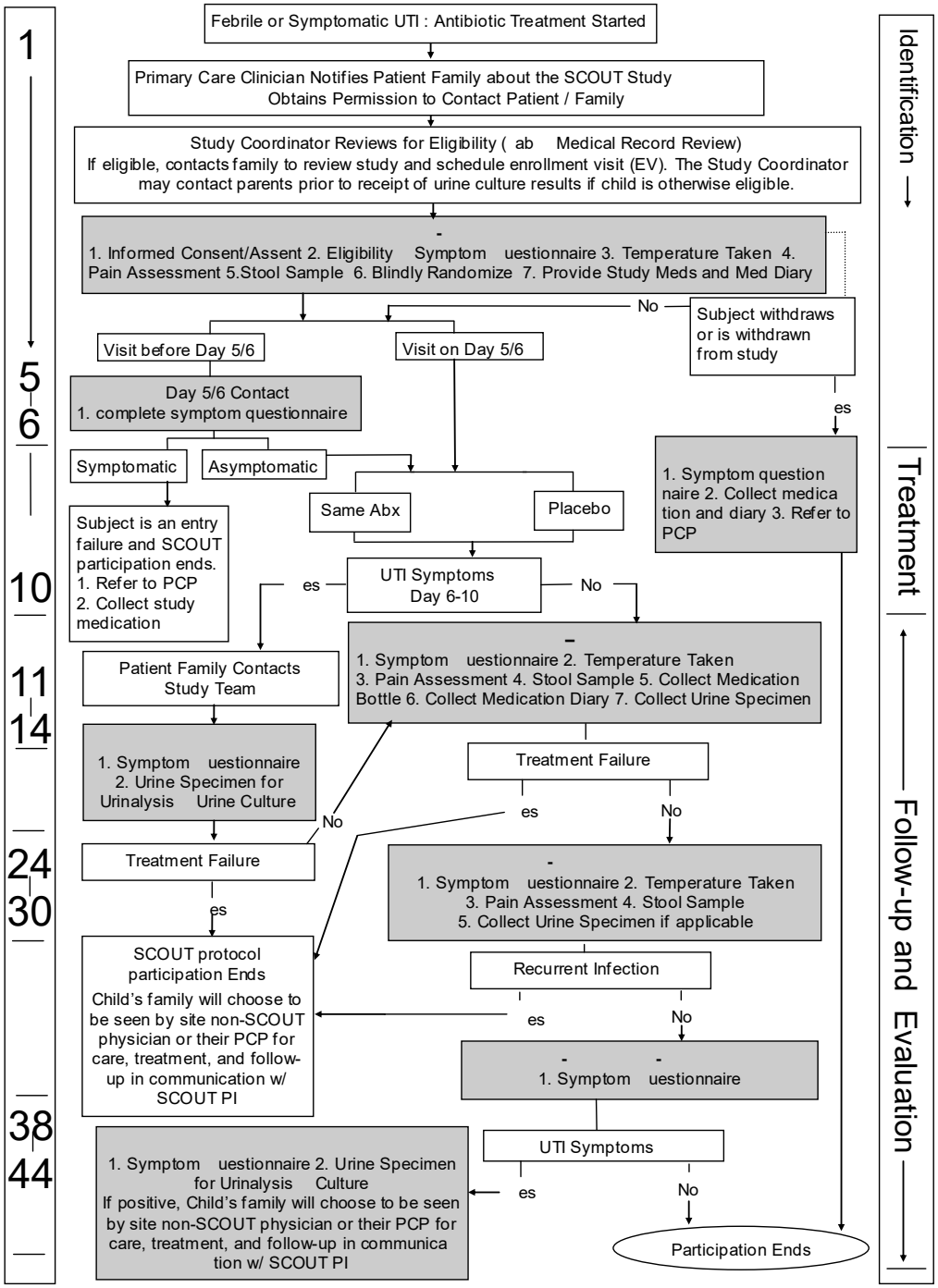
SUPPLEMENTS/APPENDICES

A. Schedule of Events

B. SCOUT UTI Timeline Definitions

C. Study product agent package inserts: Sulfatrim (TMP-SMX), Suprax (Cefixime), Cephalexin

APPENDIX A: SCHEDULE OF EVENTS



APPENDIX B: SCOUT UTI TIMELINE DEFINITIONS