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Risk Factors for and Impact of Ambulatory Urinary Tract infections Caused by High Mic-Fluoroquinolone Susceptible E. Coli in Women

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Abstract
Coincident with the increasing use of fluoroquinolones (FQs) as the first-line agent for treatment of urinary tract infections (UTIs) in adults, the prevalence of high MIC fluoroquinolone susceptible *E. coli* (high MIC-FQSEC) which are the *E. coli* isolates with reduced susceptibility to FQs has increased substantially. The high MIC-FQSEC strains may serve as an important reservoir for FQ resistance in that treatment of these organisms with a FQ has been associated with future emergence of resistance.

To establish an effective program for controlling emergence of FQ resistance, it is necessary to understand the risk factors for, and impact of infection caused by high MIC-FQSEC. To identify risk factors for high MIC-FQ susceptibility, we conducted a case-control study of female subjects with UTIs caused by FQSEC at outpatient services within University of Pennsylvania Health System, Philadelphia. A total of 1836 subjects with low MIC-FQSEC UTI (CASE) and 165 subjects with high MIC-FQSEC UTIs (CONTROL) were enrolled into our study. Independent risk factors for high MIC-FQ included Asian race, having renal diseases and previous exposure to nitrofurantoin.

To determine the impact of high MIC-FQ susceptibility, we conducted a retrospective cohort study of female subjects with ambulatory FQSEC UTIs who were treated with FQ therapy. We enrolled 246 subjects into the low MIC (unexposed) group and 29 subjects into the high MIC (exposed) group. Study subjects with high MIC-FQSEC-UTIs were approximately 8 times more likely to experience treatment failure when received FQ therapy when comparing to those with low MIC FQSEC-UTIs.

The last dissertation project was a simulation study aiming to quantitatively compare the conventional case-control (CC) approach and the novel case-case-control (CCC) approach in investigating risk factors for infection caused by FQ-resistant pathogen. Our study confirmed that the CC approach almost always overestimates the effect of previous antibiotic exposure. The difference is more pronounced if the study is to be conducted among healthy population with a lower rate of colonization and protective effect of exposure on mechanism of harboring FQ-susceptible pathogen does not exist.

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TABLE OF CONTENTS

List of tables

CONTENT-BASED PROJECTS

Pilot study

Project 1: Risk Factors for Ambulatory Urinary Tract Infections caused by High MIC-Fluoroquinolone Susceptible *E. coli* in women

Project 2: Clinical Impact of Ambulatory Urinary Tract Infections caused by High MIC- Fluoroquinolone Susceptible E. coli in women

METHOD-BASED PROJECT

Project 3: A comparison of the case-control study vs. the case-case-control study to investigate risk factors for antimicrobial resistance in simulated population

List of figures

METHOD-BASED PROJECT

Project 3: A comparison of the case-control study vs. the case-case-control study to investigate risk factors for antimicrobial resistance in simulated population

CONTENT-BASED PROJECTS

PROJECT-1 ABSTRACT

BACKGROUND: Several studies have reported an increasing prevalence of high MIC-fluoroquinolone susceptible *E. coli* (high MIC-FQSEC) which are the *E. coli* isolates with reduced susceptibility to FQ antibiotics. High MIC-FQSEC potentially results in development of fully FQ-resistance and delayed response to FQ therapy. To date, risk factors for infection caused by high MIC-FQSEC have never been successfully identified. Our study aimed to identify risk factors for ambulatory urinary tract infections (UTIs) caused by high MIC-FQSEC in women.

METHODS: We conducted a case-control study of female subjects with UTIs caused by FQSEC at outpatient services within University of Pennsylvania Health System, Philadelphia. Of subjects in whom FQSEC (a levofloxacin-MIC<4 mcg/mL) were isolated on urine culture, we included only those who met our study criteria of UTIs. Cases were subjects with UTIs caused by high MIC-FQSEC (a levofloxacin-MIC≤0.12 mcg/mL) and controls were subjects with UTIs caused by low MIC-FQSEC, (a levofloxacin-MIC>0.12 but <4 mcg/mL). Cases and controls were compared with regard to demographics, comorbid conditions, and recent use of medications (particularly antibiotics) within the 90 days prior to the UTI onset. We obtained all necessary data from HUP clinical microbiology laboratory database and Penn data store.

RESULTS: Two thousand and one female subjects with FQSEC UTIs were included from May 1, 2008 to April 30, 2011. A total of 91.8% had low MIC-FQSEC UTI while 8.2% had high MIC-FQSEC UTI. Mean age was 56.9+/-22.6 years among cases and 57.3+/-22.0 years among controls. Approximately one-fourth of subjects in both groups had at least one underlying diseases. Independent risk factors for high MIC-FQ susceptibility included Asian race [95%CI: 2.92; 1.29-6.58; p=0.02], having underlying renal diseases [95%CI: 2.18; 1.15-4.14; p=0.02] and previous exposure to nitrofurantoin [95%CI: 8.86; 1.95-40.29; p=0.04].

CONCLUSION: In addition to Asian race and having chronic renal diseases, recent use of nitrofurantoin was identified as an independent risk factor. Since this study was conducted among a relatively healthy population with a low prevalence of recent antibiotic use, we did not have

enough power to identify any associations between high MIC-FQSEC and other uncommonly used antibiotics.

PROJECT-2 ABSTRACT

BACKGROUND: Negative impact of high MIC fluoroquinolone (FQ) susceptibility on treatment response to FQ antibiotics has been clearly documented in infections caused by *Salmonella enterica* serovar Typhi (S. typhi). However, no studies have successfully determined the impact of urinary tract infections (UTIs) caused by high MIC-fluoroquinolone *E. coli* (high MIC-FQSEC) on treatment efficacy of FQ therapy.

METHODS: We conducted a retrospective cohort study of female subjects with FQSEC UTIs who received FQ therapy at outpatient services within University of Pennsylvania Health System, Philadelphia. In addition to retrieving data from the HUP clinical microbiology database and the Penn Data Store, we performed chart-review to capture all possible events of treatment failure. Exposed subjects were female subjects with high MIC-FQSEC UTIs while unexposed subjects were female subjects with low MIC-FQSEC UTIs.

RESULTS: During the 3-year of study period, we enrolled 246 subjects into the low MIC group and 29 subjects into the high MIC group. Two of the 246 subjects in the low MIC group and two of the 29 subjects in the high MIC group experienced short-term treatment failure (0.8% vs. 6.9%, p=0.06). Risk difference and risk ratio for short-term treatment failure were 0.06 [-0.03-0.15; exact-p=0.06] and 8.48 [1.24-57.97; exact-p=0.06], respectively. By adjusting with the variable of underlying cerebrovascular diseases, the Odds Ratio (OR) increased from 9.04 [95% CI=1.22-66.77; p=0.03] to 9.73 [95% CI=1.11-85.16; p=0.04]. Including the variable of having at least one underlying disease into the final model reduced the OR of the high MIC-FQ susceptibility to 8.53 [95% CI=1.14-63.96; p=0.04].

CONCLUSION: Our study was the first study demonstrating the negative impact of the high MIC-FQ susceptibility on the treatment response to FQ therapy among female subjects with ambulatory UTIs caused by *E. coli.* We believe this negative impact may be more intensified in more serious clinical situations. Future studies in other clinical settings should be conducted to fill the gap of knowledge.

BACKGROUND

In recent decades, the emergence of antimicrobial resistance has become an important issue of clinical and public health concern. Since the widespread use of fluoroquinolones (FQs) in the late 1990's, the prevalence of FQ resistance has been constantly increasing. Based on data from a U.S. study of outpatient urinary tract infections (UTIs), the prevalence of FQ resistance in *E. coli* urinary isolates was 1% in 1999 and subsequently increased to 9% in 2005.[1] Furthermore, Canadian surveillance studies revealed that the prevalence of FQ resistance among outpatient *E. coli* urinary isolates increased from approximately 1% during the 2003-2004 period[2] to 19% during the 2007- 2009 period.[3] The increasing prevalence of FQ resistance has created very real challenges for physicians and healthcare institutions to treat these resistant infections as well as to implement effective interventions for controlling emergence of antimicrobial resistance.

The problem of emergence of FQ resistance among *E. coli* urinary isolates has important public health implications for several reasons. First, infections caused by resistant pathogens are associated with higher morbidity, higher mortality and increasing in hospital expenditures.[4-8] Second, *E. coli* is the most common causative pathogen of UTIs.[9] Lastly, FQ antibiotics will lose their utility for treatment of UTIs if these trends in FQ resistance continue. Although considerable time and resources have been used to explore this problem, the gaps in knowledge still exist.

Antimicrobial resistance as a global health threat

The emergence of antimicrobial resistance has been recognized as a global health threat.[8] Acquiring a resistant pathogen does not only worsen clinical outcomes but also increases hospital expenditures.[4] A cohort study of 662 hospitalized patients at a university hospital in the U.S. reported that hospital expenditures attributable to nosocomial infections caused by resistant gram-negative bacteria were 29.3% (95% CI=16.23-42.35; P<0.001) higher than nosocomial infections caused by antibiotic-susceptible gram-negative bacteria.[6] In addition to higher antibiotic costs, an increase in overall hospital expenditures is also attributable to longer hospital stays, more laboratory and imaging tests as well as extra costs for rehabilitation services.[5-7]

The limited number of effective antimicrobial agents in the market makes this situation even more critical. Due to this antibiotic pipeline crisis, the Infectious Diseases Society of America (IDSA) recently published the inaugural statement entitled "The 10x‘20 Initiative: Pursuing a Global Commitment to Develop 10 New Antibacterial Drugs by 2020". A global commitment to urgently develop new antibacterial agents is needed to resolve this critical situation.

Fluoroquinolone (FQ) antibiotics and their properties

The first quinolone, nalidixic acid first became available in the 1960s. The first generation-quinolones were only active against gram-negative bacteria, not gram-positive bacteria.[10] Fluoroquinolones (FQs), the second generation-quinolones (norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin and levofloxacin) were developed by adding a fluorine atom at position C-6 of the quinolone molecule. This new molecular structure provided greater potency against gram-negative bacteria and moderate potency against gram-positive bacteria. The third generation-FQs (sparfloxacin, gatifloxacin and grepafloxacin) have higher potency against gram-positive bacteria (especially pneumococci) while the fourth generation-FQs (trovafloxacin, moxifloxacin, gemifloxacin, garenoxacin and sitafloxacin) have expanded activity against common gram-positive bacteria and anaerobes.[11]

FQs have promising pharmacokinetic properties including excellent bioavailability and good tissue penetration. All FQ antibiotics on the market except moxifloxacin are mainly eliminated through the kidney and all are concentration-dependent antibiotics. The most important parameter predicting the efficacy of FQ therapy is the ratio of the area under plasma concentration-curve to minimal inhibitory concentration (AUC:MIC).[11] Based on data from previous in-vitro and in-vivo studies[12-16], the AUC: MIC threshold varies across organisms and sites of infection. A clinical trial of community-acquired respiratory tract infection caused by *Streptococcus pneumoniae* reported that an AUC:MIC ratio of 33.7 or higher is associated with a microbiological eradication rate of 100%.[14] Furthermore, clinical studies evaluating the efficacy of ciprofloxacin for treatment of serious gram-negative bacterial infections found that the optimal

AUC: MIC ratio to achieve a maximum success rate and a shorter duration of bacterial eradication is ≥125.[13,15] Given these findings, maximizing the AUC:MIC ratio should provide a better opportunity to cure infection and eradicate causative pathogens.[17]

FQ antibiotics were approved for treatment of a broad range of infections including urinary tract, respiratory, gastrointestinal, genital tract, bone and joint and systemic infections.[18,19] Because of their broad spectrum-coverage, favorable pharmacokinetic properties and promising clinical efficacy, the FQ antibiotics have been recommended as first-line therapy for uncomplicated cystitis and acute pyelonephritis in women since 1999.[20] The substantial increase in FQ consumption has consequently led to emergence of FQ resistance.[21,22]

Mechanisms of FQ resistance

FQ antibiotics kill bacteria by binding to two enzymes (DNA gyrase and topoisomerase IV), resulting in disruption of the replication and transcription process of bacterial DNA.[10] FQ resistance generally emerges by point mutations in the coding regions of the DNA gyrase subunits (*gyrA* and *gyr B*) and DNA topoisomerase IV (*par C* and *par E*) in a stepwise pattern.[23] As the number of mutations increases, the MIC to FQ increases. While DNA gyrase is the primary target in gram-negative bacteria[24], topoisomerase is the primary target in most gram-positive bacteria.[25] Data from several molecular studies revealed that *E. coli* urinary isolates with reduced susceptibility to FQs typically harbor a single mutation in a *gyr*A gene while *E. coli* isolates with full resistance to FQs (FQREC) usually have double mutations in the *gyr*A and *par*C genes.[26-28] These findings confirm that stepwise mutations are necessary for development of FQ resistance.

Other less common mechanisms of FQ resistance include alterations in membrane porin production and efflux pumps.[29] Additionally, FQ resistance can be the result of the plasmid-mediated multidrug-resistant *qnr* gene which can interfere with FQs from binding to bacterial DNA.[30,31]

Minimal Inhibitory Concentration and FQ susceptibility interpretation

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of a given antibiotic that can inhibit growth of bacteria in vitro. The Clinical and Laboratory Standards Institute (CLSI)

develops the performance standards for antimicrobial susceptibility testing which have been widely used as the MIC interpretative standards in the United States and other countries worldwide.[32] Antimicrobial susceptibility is traditionally reported as susceptible, intermediately susceptible, or resistant. The MIC-cutoff value (or MIC-breakpoint) of each antibiotic is chosen based on several factors including pharmacokinetics, pharmacodynamics and data obtained from previous clinical studies of that given antibiotic.

Several FQ antibiotics (such as ciprofloxacin, levofloxacin, ofloxacin, etc.) can be used as an indicator of FQ resistance. The MIC-cutoff value varies across the type of tested antibiotic and species of microorganism. For examples, an *Enterobacteriaceae* isolate is considered FQ-susceptible (FQS) if the MIC to levofloxacin is < 4mcg/mL, FQ-intermediate susceptible (FQI) if the MIC to levofloxacin is ≥ 4 but <8 mcg/ml and FQ-resistant (FQR) if the MIC to levofloxacin is ≥ 8 mcg/ml. Additionally, the MIC to ciprofloxacin of ≤1, 2 and ≥4 mcg/ml are used as a cutoff value to document FQS, FQI and FQR strains among *Enterobacteriaceae* isolates.

In addition to these three standard susceptibility groups (FQR, FQI and FQS), some investigators further categorized the FQS *E. coli* (FQSEC) into two additional groups, based on the MIC-cutoff value; 1) Low MIC-FQSEC group (MIC level to levofloxacin ≤0.25 mcg/mL); and 2) High MIC-FQSEC group (the MIC level to levofloxacin >0.25 but <4 mcg/ml). In some studies, these two groups were also called fully susceptible strains and reduced susceptible strains, respectively.[27,33] This additional categorization is important because the high MIC-FQSEC is strongly associated with the presence of single mutation in the *gyr*A gene.[27]

Epidemiology of infections caused by FQR and high MIC-FQS pathogen

Only a few years after the introduction of FQs, emergence of FQ resistance was reported.[21,34] Since then, the prevalence of FQ resistance has been constantly increasing across bacterial organisms, sites of infection and geographical locations. Data from the 1997 SENTRY antimicrobial surveillance program revealed that the prevalence of FQ resistance among community-acquired and nosocomial bacteremia in the United States was 2.4% in *E. coli*, 1.6% in

Klebsiella spp. and 4.2% in *Enterobacter* spp.[35] The 2008-2010 SENTRY study subsequently reported the higher prevalence of FQ resistance among gram-negative bacilli isolated from Latin American medical centers. The prevalence of FQ resistance in this study was 40.2% in *E. coli*, 41.3% in *Klebsiella* spp. and 21.1% in *Enterobacter* spp group.[36] Emergence of FQ resistance has been documented in the long-term-care facility (LTCF) setting as well. A 1998 - 2003 survey of 4,954 clinical isolates from four LTCFs in U.S. revealed that the prevalence of FQ resistance in Enterobacteriaceae organisms ranged from 7.8% to 48.7%.[37]

The problem of FQ resistance is even more critical among uropathogens.[1,33,38,39] A U.S. study of outpatient UTIs reported that the prevalence of FQ resistance in *E. coli* urinary isolates was 1% in 1999 and subsequently increased to 9% in 2005.[1] The increasing prevalence of FQ resistance among *E. coli* uropathogen was also documented in Canada. Based on data from recent surveillance studies from Canada, the prevalence of FQ resistance among outpatient *E. coli* urinary isolates increased from approximately 1% in 2003-2004[2] to 19% in 2007- 2009.[3] Furthermore, a study of community-acquired UTIs conducted in Turkey revealed that 17% of *E. coli* strains isolated from uncomplicated cases and 38% of *E. coli* strains isolated from complicated UTI were resistant to FQ.[40]

In addition to the high prevalence of FQ resistance, a significant proportion of high MIC-FQSEC has been reported from several studies.[27,33] A 1998 Taiwan nationwide survey of 1,203 *E. coli* urinary isolates found that 11.3% of isolates were resistant to FQ and 21.7% of isolates were high MIC-FQS strains.[27] Data from a surveillance of fecal *E. coli* isolates at two hospitals in U.S. revealed that the prevalence of FQR isolates and high MIC-FQS isolates were 12.9% (102/789) and 5.8% (46/789), respectively.[33]

A number of studies in the past have investigated risk factors for FQ resistance across infecting organisms and sites of infection. A case-control study from U.S. community and university hospitals investigating nosocomial *E. coli* and *K. pneumoniae* infections, noted that FQ resistance was independently associated with recent FQ use (OR [95% CI] = 5.25 [1.81-15.26]), residence in a long-term care facility (3.65 [1.64-8.15]), recent aminoglycoside use (8.86 [1.71-

45.99]) and older age (1.03 [1.01-1.06]).[38] Another case-control study from a German university hospital compared 51 case patients with nosocomial FQREC infections to 102 control patients with nosocomial FQSEC infections. Independent risk factors for FQ resistance from this study were prior FQ therapy (OR [95% CI] =18.49 [5.53-61.82]), urinary tract abnormalities (6.69 [1.68-26.63]) and prior therapy with other antimicrobial agents (3.57 [1.38-9.27]).[41] During 1995-2002, a prospective cohort study of invasive pneumococcal infection was conducted to investigate epidemiology of antimicrobial resistance. This study found that infection with FQR pneumococci was significantly associated with previous FQ use (OR [95% CI] = 12.10 [4.22-35.40]), current residence in a nursing home (12.9 [3.95-43.9]) and nosocomial acquisition of pneumococcal infection (9.94 [2.22-44.60]).[42]

Risk factors for FQ resistance in a specific site of infection such as UTI have also been investigated in many studies.[43-45] A case-control study comparing 136 case patients with FQR gram-negative UTIs to 139 control patients with FQS gram-negative UTIs found a strong association between FQ resistance with several factors including recent exposure to beta-lactamase inhibitors (OR [95% CI] =14.98 [2.92-76.99]), extended spectrum cephalosporins (9.82 [3.37-28.60]), FQs (5.36 [2.20-13.05]) and clindamycin (13.90 [1.21-10.49]).[44] A 2004 study of community-acquired UTIs revealed that UTIs caused by FQREC were strongly associated with previous exposure to FQs (OR [95% CI] = 30.35 [5.82-158.42]) and recurrent UTIs (8.13 [2.95-22.37]).[43] Recently, a nested case-control study was conducted to assess risk factors for FQ resistance in community-onset febrile *E. coli* UTIs. This study documented recent exposure to FQ (OR [95% CI] =17.5 [6.0-50.7]) and recent hospitalization (2.0 [1.0-4.3]) as independent risk factors for FQ resistance.[45] In summary, recent FQ exposure has been documented as an independent risk factor for FQ resistance across infecting organisms, sites of infection and study settings (community hospital, university hospital and ambulatory setting).[38,40,41,43-50]

While a substantial number of studies focused on risk factors for infections caused by FQR pathogens, knowledge of risk factors for infections caused by high MIC-FQS pathogens is very limited. To our knowledge, the 1998 Taiwan-nationwide survey was the only study that

investigated risk factors for infection caused by high MIC-FQSEC.[27] This study included 1,203 clinical isolates of *E. coli* from all sites of infection, all age groups and both outpatient and inpatient settings. They subsequently compared characteristics of 19 patients with high MIC-FQSEC infection to 57 patients with low MIC-FQSEC infection. This study did not detect any significant association between the high MIC-FQ susceptibility and patients' demographics, underlying diseases, length of hospitalization, presence of invasive catheter or recent exposure to antimicrobial therapy. This was most likely related to the very small sample size. Another important limitation of this study was the heterogeneity of study population. This study included isolates from all anatomic infection sites and both inpatient and outpatient settings while risk factors for high MIC-FQSEC infections may differ across anatomic sites and clinical settings. Thus far, the risk factors for infections caused by high MIC-FQSEC have never been successfully identified.

Impact of FQ susceptibility on efficacy of FQ therapy

Over the past decade**,** the prevalence of FQ resistance among uropathogens has been constantly increasing.[1,51] Decrease in treatment efficacy of FQ therapy has been noted in several studies of *E. coli* UTIs.[52,53] According to data from clinical studies conducted in late 80's, short course FQ regimens for treatment of uncomplicated UTIs provided an approximately 95% clinical cure rate and 90% microbiological cure rate.[54-57] In a recent clinical trial of uncomplicated UTI which was conducted in an area with a relatively high prevalence of FQREC (>10%), a 3-day norfloxacin regimen (400 mg twice a day for 3 days) achieved only a 76% complete response rate.[53] In another recent study of uncomplicated UTIs, a short course gatifloxacin regimen (200 mg once daily for 3 days) provided only a 93% overall clinical cure rate (95% in susceptible cases and 75% in resistant cases).[52]

Due to the increased prevalence of FQ resistance among uropathogens, the international clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women was updated by the IDSA and the European Society of Microbiology and Infectious

Diseases (ECMID) in 2011.[58] They recommend using alternative antimicrobials (such as nitrofurantoin or trimethoprim-sulfamethoxazole) for treatment of uncomplicated cystitis. FQ therapy should be reserved only for patients with acute pyelonephritis in areas in which the prevalence of FQ resistance is not known to exceed 10% (A-I).

The negative impact of high MIC-FQ susceptibility on treatment outcomes has been previously documented in several studies. However, the majority of these studies specifically focused only on infections caused by *Salmonella enterica* serovar Typhi (S. typhi).[59,60] An association between reduced susceptibility to ofloxacin and clinical outcome in patients with enteric fever who received ofloxacin has been clearly demonstrated in a recent publication from Vietnam.[59] This study obtained individual patient data from 7 open randomized controlled trials of antibiotic therapy among subjects with enteric fever in Vietnam. A total of 540 subjects with enteric fever who were treated with ofloxacin were included while subjects who received other types of antibiotic were excluded. The treatment success rate among subjects with infection caused by high MIC-FQS S. Typhi (a MIC to ofloxacin 0.25-0.50 mg/L) was only 73% while the treatment success rate among subjects with infection caused by low MIC-FQS S. Typhi (a MIC to ofloxacin ≤0.125 mg/L) was 96%. In addition to a lower treatment success rate, subjects with infection caused by high MIC-FQS S. Typhi were more likely to receive a higher dosage of ofloxacin and/or a longer duration of therapy.

Furthermore, the delay in treatment response to FQ therapy was noted in a case series of patients with infection caused by high MIC-FQS S. Typhi and S. Paratyphi [60] Among 21 patients who were infected with S. Typhi or S. Paratyphi, seven of them (33%) were infected with high MIC-FQS strains (a MIC to ciprofloxacin 0.125–0.5 mg/L). Of these 7 subjects with the high MIC-FQS strain, 70% of them (5 /7) were initially treated with oral ciprofloxacin and 80% of subjects who received oral ciprofloxacin (4/5) subsequently required intravenous FQ therapy or switching to other parenteral antibiotics.

To date, no studies have determined the association between high MIC-FQ susceptibility and treatment efficacy of FQs among subject with UTI caused by *E. coli*. According to the

concept of optimal AUC:MIC of FQ antibiotics, maximizing the AUC:MIC ratio offers a better opportunity to cure infection and eradicate causative pathogens.[17] Therefore, it is reasonable to believe that the FQ treatment failure might be higher among subjects with high MIC-FQSEC UTIs when comparing to those with low MIC-FQSEC UTIs. However, this assumption has never been sufficiently proved in any in-vivo or in-vitro study.

Therefore, we conducted two separate studies to explore these issues. The first project was a case-control study aiming to investigate risk factors for ambulatory UTIs caused by high MIC-FQSEC *E. coli* in women. The second project was a retrospective cohort study aiming to investigate the clinical impact of high MIC-FQSEC UTI on clinical outcomes. Study subjects of project-2 were all subjects from the project-1 who received FQ therapy. Since there is no standard definition for ambulatory UTIs, we conducted a pilot study to determine the appropriate study definition to identify UTIs before pursuing these two projects. Furthermore, the results from this pilot study could be used to accurately estimate an achievable sample size for our proposed projects.

PILOT STUDY

1. RATIONALE OF THE PILOT STUDY

To date, only a few definitions for UTIs have been widely accepted in biomedical research. The most well-known one is the surveillance definition for healthcare-associated UTIs established by the Centers for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN). The first version of the CDC/NHSN definition was introduced in 1988[61] and then updated in 2008.[62] The CDC/NHSN definition has been widely used in epidemiological research, however it was specifically designed to identify healthcare-associated UTIs not ambulatory UTIs. Details of this definition are shown in table 1.

Another well-known definition was proposed by the IDSA in 1992.[63] This definition can be used to identify UTI episodes in various clinical settings (ambulatory UTI, healthcare-associated UTI, etc.). However, this definition was exclusively developed to evaluate new antimicrobial agents in clinical studies. Therefore, the majority of its criteria are clinical based and not suitable for studies using an electronic database.

In 2010, Landers et al conducted a study evaluating the ICD-9 coding algorithm to detect UTI in electronic data at a 745-bed tertiary hospital in New York.[64] They compared several computer-based decision rules including combinations of laboratory data (culture reports and urinalysis findings), patient clinical data (fever), and administrative data (ICD-9 codes). The ICD-9 codes include; 599.0 (Urinary tract infection, site not specified); 590.x (Infection of kidney); 595.0 (Acute cystitis); 597.x (Urethritis, not sexually transmitted diseases). The ICD-coding algorithm reached only 55.6% sensitivity and 93.9% specificity when compared to combinations of urine culture and symptom-based definition. In addition to the poor sensitivity, this coding algorithm was specifically designed to detect UTI in the inpatient setting. Therefore, the Landers's algorithm is not suitable for our study.

To our knowledge, a standard definition of UTIs in the ambulatory setting has never been proposed. Therefore, a well-designed study to identify a standard definition of ambulatory UTIs was needed. Our pilot study had two specific aims;

1) To establish a study definition to identify UTIs in the ambulatory setting,

2) To accurately estimate an achievable sample size for our proposed projects.

2. METHODS

The pilot study was conducted during Feb 1-April 30, 2011. We obtained a list of ambulatory subjects with significant *E. coli* bacteriuria ($\geq 10^5$cfu/ml for female and $\geq 10^4$cfu/ml for male) from the clinical microbiology laboratory at the Hospital of University of Pennsylvania (HUP). This laboratory serves more than 70% of clinical practices within the University of Pennsylvania Health System.

We randomly sampled 100 subjects (all women) with significant *E. coli* bacteriuria during the study period. All subjects' medical records were obtained via the EPIC/Medview system. To evaluate the applicability of the CDC/NHSN definition (table 1) and the ICD-9 coding algorithm to ambulatory subjects with FQSEC bacteriuria, we applied these two definitions to data that we obtained from EPIC/Medview. The written diagnosis of UTI on patients' medical record was considered a gold standard.

3. RESULTS

During the 3-month study period, 1,357 subjects with significant *E. coli* bacteriuria were identified, 1,006 (74.1%) subjects with low MIC-FQSEC bacteriuria, 90 (6.6%) subjects with high MIC-FQSEC bacteriuria, 8 (0.6%) subjects with FQIEC bacteriuria and 253 (18.6%) subjects with FQREC bacteriuria. Among 1096 subjects with significant FQSEC bacteriuria, a total of 930 (84.9 %) subjects were female. Subsequently, we randomly sampled 100 subjects from these female subjects with FQSEC bacteriuria (low and high MIC-FQSEC bacteriuria).

Among the 100 subjects who were enrolled into our pilot study, 3 subjects came from practices that have not yet employed the EPIC/Medview system. Thus, only 97 subjects had

available electronic medical records on the EPIC/Medview database for review. Of these 97 subjects with available medical records, 90 subjects had a written diagnosis of UTI on their electronic medical record. Detail and Test characteristics of each criterion and the combined criteria are shown in the table 2.

By using the C.4 criteria (combination of SS, DX and LAB criteria), we were able to capture 79 subjects with UTI and only one subject without UTI was falsely identified as having UTI. This C.4 criterion provided us the best discrimination ability (sensitivity=87.8.0% and specificity=85.7%) when comparing to all other combined criteria.

4. DISCUSSION

Based on the study results, the C.4 criteria provided the best discrimination ability. If we apply the C.4 criteria to all female subjects with significant FQSEC bacteriuria on the list from the HUP microbiology laboratory database, it is estimated that approximately 70% of these subjects were diagnosed with FQSEC UTI.

5. TABLES

Table 1. The CDC/NHSN definition for healthcare-associated UTIs

To be diagnosed of UTI, eligible subjects must meet **both criterion 1 and criterion 2.**	
Criterion 1	**The subject has a positive urine culture** $\geq 10^5$cfu/ml, with no more than two species of microorganisms
Criterion 2	**At least one of the following** a) Dipstick test positive for leukocyte esterase and/or nitrate b) Pyuria ($\geq$10 white blood cells (wbc) /mm^3 or $\geq$ 3 wbc /high power field of unspun urine) c) Physician diagnosis of a urinary tract infection d) Physician institutes appropriate therapy for UTI within 2 days of the culture date.[20,58] Appropriate antibiotic regimens are shown below. ▪ Fluoroquinolones ▪ Nitrofurantoin ▪ Sulfamethoxazole-trimethoprim ▪ Fosfomycin ▪ Beta-Lactam agents, including amoxicillin-clavulanate, cefdinir, cefaclor, and cefpodoxime

Table 2. Sensitivity and Specificity of each criterion and combined criteria for UTIs

Criteria	Sensitivity	Specificity
A. CDC/NHSN definitions		
A.1 At least one sign and symptom criteria (SS) ▪ ICD-9 code of urgency ▪ ICD-9 code of frequency ▪ ICD-9 code of dysuria ▪ ICD-9 code of suprapubic tenderness	25.6% (23/90)	85.7% (6/7)
A.2 At least one laboratory criteria (LAB) ▪ Dipstick test positive for leukocyte esterase ▪ Pyuria (>10 wbc/mm3 or > 3 wbc/HPF of unspun urine)	21.7% (20/90)	85.7% (6/7)
A.3 Physician institutes appropriate therapy for UTI within 2 days of the culture date (RX)	58.9% (53/90)	85.7% (6/7)
B. At least one ICD diagnosis code of UTIs (DX) ▪ 599.0 Urinary tract infection, site not specified ▪ 590.x Infection of kidney ▪ 595.0 Acute cystitis ▪ 597.x Urethritis, not sexually transmitted diseases	66.7% (60/90)	100% (7/7)
C. Combined criteria		
C.1 SS or LAB	40.0%(36/90)	85.7%(6/7)
C.2 SS or DX	82.2%(74/90)	85.7%(6/7)
C.3 LAB or DX	75.6% (68/90)	85.7%(6/7)
C.4 SS or LAB or DX	87.8% (79/90)	85.7% (6/7)
C.5 SS or LAB or DX or RX	94.4% (85/90)	71.4%(5/7)

PROJECT 1: Risk factors for ambulatory urinary tract infections caused by high MIC- fluoroquinolone susceptible *E. coli* in women

1. METHODS

We conducted a case-control study of female subjects with ambulatory UTIs caused by FQSEC from May 1, 2008 to April 30, 2011. The study was approved by the University of Pennsylvania Institutional Review Board.

1.1 Study setting

The study was conducted at outpatient practices within the University of Pennsylvania Health System (UPHS). The UPHS network consists of a broad range of healthcare facilities including one university hospital, two community hospitals, two specialty centers, six community practices and a number of outpatient clinical practices within the Clinical Care Associates (CCA) and within the Clinical Practices of the University of Pennsylvania (CPUP). The CCA is a network of single and group primary care practices owned by UPHS as well as dozens of hospital and community based practices in both primary care and subspecialty medicine. CPUP is an integral part of Penn Medicine, with a faculty practice plan and multispecialty satellite facilities providing over two million outpatient visits per year.

Since we identified eligible subjects through the database of the clinical microbiology laboratory at the Hospital of University of Pennsylvania (HUP), only practices that sent their clinical specimens for processing at the HUP microbiology laboratory during the study period were eligible for the study. Additionally, our major source of data was EPIC/MEDVIEW care (an electronic medical record system). Therefore only subjects from clinical practices that employed the EPIC/MEDVIEW system during the study period were eligible for the study.

The UPHS network provides health services to the population who live within the greater Philadelphia area. Based on 2010 US census data, Philadelphia county has approximately 1.5 million residents, in whom 47% are African-American, 42% White and 6.3% Asian.

1.2 Study subjects

1) Adult female subjects (age ≥ 18 years)
2) Had a positive urine culture for FQSEC (detail shown in the section 1.3.1)
3) Met the study definition for UTIs (detail shown in the section 1.3.2)
4) First episode of UTI during the study period (detail shown in the section 1.3.3)
5) Registered to the UPHS system for at least 3 months prior to the index date

1.3 Study definitions

Subjects who met the following inclusion criteria were eligible for our study;

1) **Susceptibility to FQs**[65]

   Microbiological tests were routinely processed at the clinical microbiology laboratory located at HUP. All tests were processed by the Vitek-2 system, according to the performance standards for antimicrobial susceptibility testing established by Clinical and Laboratory Standards Institute (CLSI). Levofloxacin was used as an indicator of resistance to the FQ class of antibiotics. An isolate was considered FQ resistant (FQR) if the minimum inhibitory concentration (MIC) to levofloxacin was ≥ 8 mcg/ml. An isolate with MIC to levofloxacin < 4mcg/mL was considered FQ susceptible (FQS) while an isolate with an MIC to levofloxacin ≥ 4 but <8 mcg/ml was considered FQ intermediate susceptible (FQI). In addition, FQS urinary isolates were categorized into two groups; 1) **high MIC-FQS,** a FQS isolate with a MIC to levofloxacin ≤0.12; 2) **low MIC-FQS**, a FQS isolate with MIC to levofloxacin > 0.12 but < 4 mcg/mL.

2) **Index date:** The date that a urine specimen with FQSEC was collected.

3) **Identification of subjects with *E. coli* UTI**

   All potential study subjects for whom a urine culture grew *E. coli* were identified through the HUP clinical microbiology laboratory database. By using data retrieved from the EPIC/Medview database, only those who met our criteria for UTI within 7 days before or 7 days after the index date were considered as having *E. coli* UTI. Since no standard definition for ambulatory UTIs has been established, we created our own study definition

by combining the CDC/NHSN surveillance definition of healthcare-associated UTIs and the ICD-9 coding algorithm to detect UTI in an electronic database based on our pilot study.[62,64] Our study definition for UTIs is shown in table 3. Based on data from our pilot study, our study definition provided 87.8% sensitivity and 85.7% specificity to identify female subjects with *E. coli* UTI within our source population.

4) Identification of subjects with the first episode of UTI

It is well known that a recurrent UTI is different from a new onset UTI in various aspects including risk factors, clinical course and outcomes.[66-68] Therefore, we included only the first episode of UTI if a given patient had more than one episode of UTI during the study period. Subjects who had a UTI episode within 30 days prior to the beginning of the study were also excluded.

Furthermore, some subjects might have a UTI episode but did not have a positive urine culture due to several reasons (e.g. phone visit, no specimen collected, etc.). These UTI episodes were not captured by our study definition for UTIs and could lead to misclassifying a recurrent UTI as a new onset UTI. To reduce this problem, patients' data within 30 days prior to the index date was also reviewed. If at least one item of the criterion-2 was documented, that given episode of UTI was considered recurrent and removed from the study.

5) Identification of cases and controls

A subject in whom an *E. coli* urinary isolate was high MIC-FQS (the MIC to levofloxacin > 0.25 but < 4 mcg/mL) was considered a **case.** A subject in whom an *E. coli* urinary isolate was low MIC-FQS (the MIC to levofloxacin ≤ 0.25 mcg/mL) was considered a **control**. All eligible cases and controls were included without using any matching procedure.

1.4 Data collection

1) Data sources

We obtained all necessary data from two major sources: HUP clinical microbiology laboratory database, and Penn Data Store. The Penn Data Store is an internally developed virtual data warehouse which allows researchers to combine data from all key systems within UPHS. The list of key systems is shown below.

a. **EpicCare**, an ambulatory electronic medical record (EMR) system which includes data from all outpatient practices within UPHS
b. **Sunrise,** a Clinical Manager CPOE system and Sunrise Pharmacy (SRx) which includes inpatient order entry from all hospitals within UPHS
c. **Emtrac**, an EMR system which includes data from all encounters at an Emergency Department
d. **Cerner Millennium Laboratory Information System,** an updated laboratory database
e. **MedView**, a web-based medical record aggregator

The data elements provided by each source are summarized below (Table 4).

2) Data to be collected

Data on potential risk factors including age, race, clinic site and service (e.g., medicine, surgery), designated primary care provider, previous hospitalization, comorbid conditions, previous and current medications used and microbiological results were obtained from Penn data Store.

The presence of comorbid conditions was evaluated by applying the Enhanced ICD-9-CM coding algorithms to ICD-9 codes.[69,70] Comorbid conditions included myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular diseases, dementia, chronic obstructive pulmonary disease (COPD), liver disease, diabetes mellitus (with or without complications), chronic renal disease, malignancy and HIV infection.

Outpatient and inpatient medications used within 90 days prior and after the index date were evaluated. We recorded data of the following medications including

antibiotics, steroids and other immunosuppressive agents. The antibiotic name, antibiotic category, as well as amount of antibiotic therapy were documented. Amount of antibiotic consumption was recorded in terms of the total number of antibiotic-days (defined as the sum of exposure to each antibiotic over the time period).

Our primary risk factor of interest was previous FQ exposure within the past 3 months prior to the index date. Given that receiving FQ therapy prior to the time of culture can inhibit growth of FQ susceptible organisms, including very recent FQ therapy prior to the index date as the exposure may overestimate the risk effect of FQ antibiotic. Therefore, we **excluded** FQ antibiotics prescribed within **7 days** prior to the index date to avoid overestimation of the effect of FQ exposure. Previous exposure to all other antibiotics was defined in the same way.

Urine culture results within 90 days prior and after the index date were also obtained. Obtained data included the date of obtaining specimen, the infecting organism, the number of isolated colony forming unit, the susceptibility profile as well as the minimal inhibitory concentration (MIC).

3) Data verification

If some study subjects may seek medical care at off-network sites, this may result in underestimation of previous antibiotic exposure. To assess for this possibility, we randomly selected a total of 200 of the eligible subjects for chart-review. Any encounters within 3 months before and after the index visit were reviewed to identify documentation of off-network visits (as noted in the Penn Medical Record). Of those subjects with documented off- network visit, data on previous antibiotic exposure during that off-network visit was obtained. We found that only three of the 200 subjects had at least one off-network visit within 3 months before or after the index date but all visits happened after the index date. Therefore, we believe that the off-network antibiotic therapy was minimal.

4) Data Management

Data from all sources were stored in a computerized database management system. All information were kept strictly confidential. All data were de-identified and coded with a unique identifier that is linked to his or her identity key before starting analysis. All electronic data were kept on a password protect computer while all paper documents were stored in a locked file cabinet at the study center. Only the principal investigator had an access to the identity key.

The HUP central microbiological laboratory and the Penn Data Store provided us the dataset from their existing data management system. These prevented errors that may occur while performing the data entry. All information from chart-review were recorded by using the data abstraction form. All entries were printed legibly in black ink. In case there is missing data, the explanations were added at the end of the abstraction form. Data entry was performed by the principal investigator.

1.5 Statistical analysis

1) Introduction

First, we described the data distribution of each collected variable. Cases and controls were characterized by all potential risk factors including demographic variables, clinic site and service, comorbid conditions, previous medications used 3 months prior to the enrollment. Categorical variables were summarized by frequency while continuous variables were summarized by the mean, median, standard deviation, and range. For all calculations, a two-tailed P value of <0.05 was considered statistically significant. All calculations were performed using the STATA version 12.0 (Stata Corp, College Station TX).

2) Descriptive analysis

We characterized case and control subjects by all characteristics including demographic variables, clinic site and service, comorbid conditions; previous medications used 3 months prior to the enrollment. Categorical variables were summarized by frequency

while continuous variables were summarized by mean, median, standard deviation, and range as appropriate.

3) Bivariable analysis

Risk factors for high MIC-FQ susceptibility were thoroughly investigated by comparing characteristics of cases to controls. Our primary risk factor was previous exposure to FQ antibiotics within 3 months prior to the onset of infection. Bivariable analysis was performed to determine the unadjusted association between all potential risk factors and high MIC-FQ susceptibility. Categorical variables were compared using the chi-square or Fisher's exact test while continuous variables were compared using the student's t or Mann-Whitney U test, depending on the sample distribution. An unadjusted odds ratio (OR) and its confidence interval (CI) were reported.

4) Stratified analysis

Stratified analysis by Mantel-Haenszel test was subsequently performed to evaluate the effects of each variable of interest as a possible confounder as well as a possible interaction. The stratifying variables included year of enrollment, practice type (primary, secondary or tertiary care practices), previous hospitalization, age group, and presence of comorbid conditions. The presence of confounding was documented if a difference between the crude OR and the summary OR was more than 15%. Effect modification was assumed if the test for heterogeneity between the OR for different strata reached statistical significance ($P<0.05$).

5) Multivariable analysis

Multivariable analysis was performed by building an explanatory multiple logistic regression model. Forward-backward stepwise approach was used for selection of variables in the explanatory final model. Building of the multivariable model started with inclusion of our primary exposure variable (previous FQ exposure) regardless of its p-value. Other variables were considered for inclusion in a multivariable model if their bivariable p-value < 0.20. In addition, we also included other variables if they were found

to a confounder on stratified analysis. These potential confounders were kept in the model if they changed the effect estimate of previous FQ exposure by 15% or more. Finally, we also investigated the possible presence of interaction between variables.

6) Sample size and power calculation

Based on preliminary data from the HUP clinical laboratory database, approximately 2,700 low MIC-FQSEC and 300 high MIC-FQSEC urinary isolates were identified in 2010. Data from our pilot study (as previously mentioned) revealed that approximately 70% of these urinary isolates would be eligible for our study. Therefore, we anticipated that we would be able to enroll at least 5,600 cases and 560 controls to our study during 3 years of study period. Details of sample size calculation is shown in table 5. We believe that it would be clinically meaningful to detect any risk factors with an odds ratio (OR) equal to 2.0 or more. By using an alpha=0.05, we would have ≥ 99% power to identify any clinically meaningful risk factors. Details of the power calculation are shown in table 6.

Unfortunately, the exact sample size was remarkably lower than expected. We could enroll a total of 2,001 female subjects with non-recurrent FQSEC UTIs (165 subjects in the high MIC-group and 1,836 in the low MIC-group. However, based on this smaller sample size, we still had more than 80% power to detect any risk factors with an odd ratio (OR) equal to 2.0 or more if the baseline prevalence is >10% as shown in table 7.

2. RESULTS

During the 3-year study period, there were 11,287 urine specimens that grew *E. coli* $\geq 10^5$cfu/ml. Of these 11,287 urine specimens, approximately one-third of them (n=3,418) were obtained from subjects who met the definition for non-recurrent episode of UTI. Among those with non-recurrent episodes of *E. coli* UTI, seventy-eight percent were female (2,669 from 3,418). When we focused only on non-recurrent episodes of *E. coli* UTI, 74.9% were in the FQSEC group, 0.4% in the FQIEC group and 24.7% in the FQREC group. Of these female subjects with FQSEC UTI

(n=2,001), a total of 91.8% (1,836/2,001) had low MIC-FQSEC UTI and 8.2% (164/2001) had high MIC-FQSEC UTI.

As mentioned previously, all female subjects with non-recurrent FQSEC UTI were enrolled into our study. The female subjects with low MIC-FQSEC UTI were considered the cases (n=165) while the female subjects with high MIC-FQSEC UTI were considered the controls (n=1,836). Details of the susceptibility distribution are shown in table 8.

Demographics, diagnosis criteria for UTI and comorbid conditions of cases and controls are shown in table 9. Mean age and calendar year of enrollment were comparable between cases and controls. Asian race was significantly associated with high MIC-FQSEC in bivariable analysis [95%CI: 2.52; 1.25-5.07; p=0.02]. When comparing the diagnosis criteria for UTI between two groups, the ICD-9 code for UTI was significantly prevalent among cases [95%CI: 1.48; 1.02-2.19; p=0.03]. Regarding to the comorbid conditions, cases were more likely to have congestive heart failure [95%CI: 2.44; 1.29-4.34; p=0.001] and renal diseases [95%CI: 2.05; 1.00-3.93; p=0.02]. Furthermore, the high MIC group was borderline associated with having acute myocardial infarction [95%CI: 1.77; 0.66-4.05; p=0.16].

By comparing previous drug exposure between two groups, the high MIC group was more likely to be previously exposed to overall antibiotics [95%CI: 1.80; 0.67-4.12;p=0.15], nitrofurantoin specifically [95%CI: 5.65; 0.51-39.73; p=0.08] and H2 blocker agents [95% CI: 2.12; 0.39-7.51; p=0.20]. However, these findings did not reach statistical significance in bivariable analysis. Details of previous drug exposure within 90 days prior to the index date are shown in table 10.

The variables that remained independent risk factors for high MIC-FQSEC UTI after multivariable analysis are shown in 11. Independent risk factors for high MIC-FQ susceptibility included Asian race [95%CI: 2.92; 1.29-6.58; p=0.02], having renal disease [95%CI: 2.18; 1.15-4.14; p=0.02] and previous exposure to nitrofurantoin [95%CI: 8.86; 1.95-40.29; p=0.04].

3. DISCUSSION

Our study revealed that the distribution of FQ susceptibility is similar across clinical settings. Approximately one-fourth of isolates from subjects with *E. coli* bacteriuria, subjects with non-recurrent episode of *E. coli* UTI and female subjects with non recurrent episode of *E. coli* UTI were resistant to FQs. The remaining three-fourth of isolates from these three subgroups were susceptible to FQs. Of these susceptible isolates, less than one percent were considered high MIC-FQSEC. The prevalence of high MIC-FQSEC documented in our study was relatively low (6.1%) while the prevalence of FQREC was relatively high (24.7%) when compared to data from the Taiwan surveillance study of *E. coli* conducted in 1998[27]. Unlike our study, the Taiwan study did not exclude colonization cases or recurrent cases. Another important difference is the Taiwan study obtained clinical isolates of *E. coli* from both inpatient and outpatient settings.

One of the independent risk factors for high MIC-FQSEC UTI discovered in our study was Asian race. [44]Racial disparity has been described in various infections caused by antibiotic-resistant pathogens.[44,71,72] For instance, a recent study conducted at University of Pennsylvania revealed that African-American race was an independent risk factor for healthcare-acquired UTIs caused by FQR gram-negative bacilli.[44] However, the association between Asian race and antibiotic resistance has never been documented. It is unclear how to explain this observed relationship. We hypothesize that this may be the result of differences in amount of antibiotic consumption, pharmacokinetic/pharmacodynamics of antibiotics, or dietary patterns. It is well known that exposure to a subtherapeutic level of antibiotic is associated emergence of antibiotic resistance.[73,74] Because Asian people tend to have a smaller body size, elimination of an antibiotic may take time longer and lead to prolonged exposure to subtherapeutic level of antibiotic. In addition to direct exposure of antibiotic, antibiotic use in food production is also an important source of non-therapeutic antibiotic exposure. Given that antibiotic use in agriculture and livestock can result in emergence of antibiotic resistance[75,76], it is reasonable to believe that some dietary patterns may be a risk factor for acquiring antibiotic-resistant pathogens. However,

we could not explain why this specifically affects only Asian subjects, therefore further study exploring the racial differences should be performed.

Renal disease was also identified as an independent risk factor for high MIC-FQSEC UTI. The correlation between the renal disease and several types of antibiotic resistance has already been documented[77-79]. In addition, it is well-known that patients with renal diseases are more likely to receive antibiotics especially urinary anti-infective agents.

Nitrofurantoin was found to be an independent risk factor for high MIC-FQSEC UTI.. Given that the only approved indication of nitrofurantoin is for the treatment of acute uncomplicated UTI (acute cystitis) caused by susceptible strains of *E. coli* or *Staphylococcus saprophyticus, we* hypothesized that subjects without *E. coli* bacteriuria but with signs and symptoms of UTI may be treated with nitrofurantoin. SImilarly, subjects with *E. coli* bacteriuria but had no sign and symptom of UTI may be treated with nitrofurantoin as well. To evaluate this hypothesis, we further extracted data from electronic medical records of all study subjects who were previously exposed to nitrofurantoin (n=6). Of these six subjects who had history of previous nitrofurantoin exposure (n=4 in the low MIC group and n=2 in the high MIC group), three in the low MIC group and none in the high MIC group had at least one episode of *E. coli* bacteriuria prior to the index date. However, these 3 subjects did not meet our study definition for UTIs and would thus not have been excluded based on a possible recurrent UTI Given our approach to identification of eligible study subjects (based on a positive urine culture) it is impossible to know how many subjects with sign and symptom of UTI were treated with nitrofurantoin without documented *E. coli* bacteriuria.

Surprisingly, we could not detect any significant association between high MIC-FQSEC UTI and other antibiotics except nitrofurantoin. Since we enrolled all study subjects from the ambulatory setting, the majority of them had no comorbidities and no history of previous antibiotic exposure. Given this very low prevalence of antibiotic exposure among our study subjects, a small association may be missed. We also tested a new capture period for previous antibiotic exposure to see whether the prevalence of previous antibiotic exposure would differ. As noted previously, excluded antibiotic use in the 7 days prior to the UTI from consideration as a risk

factor (assuming this antibiotic use reflected early therapy for the UTI that would be identified shortly). In a secondary analysis, we changed this exclusion period to 3 days, rather than 7 days. However, the previous antibiotic exposure was unchanged.

Our study has numerous strengths. To our knowledge, this was the first study which was specifically designed to investigate risk factors for ambulatory UTIs caused by high MIC-FQSEC. Since risk factors for developing high-MIC susceptibility might be different across sites of infection, causative organisms and clinical settings (inpatient, outpatient or long-term care facility), therefore our study focused exclusively on ambulatory UTI caused by FQSEC in women. We also excluded all recurrent episodes of UTIs because they may be different from non-recurrent UTIs. Unlike the previous study that failed to distinguish true infection from colonization[27], our study enrolled only subjects with documented FQSEC-UTI. Furthermore, we carefully performed a pilot study to select the most accurate diagnosis criteria for ambulatory UTIs because the standard definition for UTIs in the ambulatory setting has never been established.

Our large study sample size is considered the strength as well. Even our study had the smaller-than-expected sample size but it was still the largest study that primarily focused on high MIC-FQSEC UTI. Based on a total sample size of 2,001 subjects (165 cases vs. 1,836 controls), we had more than 80% power to detect any risk factors with an OR of 2 or more if the exposure prevalence in the control group ranges between 0.1 to 0.9. Therefore, we believe that our study had an adequate power to identify any clinically meaningful risk factors for high MIC- FQEC UTIs.

Our study also has some potential limitations. A major concern in a case-control study is selection bias. Selection bias occurs when a probability of being selected to be either the case or the control is related to some specific factors other than having the disease. Since our study definition of UTI mainly relied on urine culture results, combined with data from the Penn Data Store and the HUP microbiology laboratory database, this might be a source of selection bias. To minimize this problem, we also performed the pilot study to identify the most accurate diagnosis

criteria for ambulatory UTIs. We used the diagnosis criteria that have been shown to provide good discrimination ability (sensitivity=87.8% and specificity=85.7%).

Although we used the validated criteria to identify subjects with FQSEC UTIs, the potential for selection bias still exists. For example, urine examination and urine culture are more likely to be requested in subjects with previous history of UTI. Furthermore, some patients with UTI may not seek medical attention or their attending physician does not request urine culture. Therefore, sicker patients are more likely to be diagnosed UTI by our study definition. However, there is no reason to believe that this bias would be differential between cases and controls.

Another potential limitation in nearly all case-control studies is misclassification bias. Although the case or control status was unlikely to be misclassified, the exposure may be underestimated. Since the exposure of interest was only obtained through the Penn Data Store, we could not capture any antibiotic prescriptions outside UPHS. To evaluate the magnitude of this issue (as described previously), we randomly selected 200 subjects and performed chart-review to evaluate how many subjects had a history of an off-network visit. Of these 200 subjects, only 3 of them (1.5%) had at least one off-network visit within 3 months before or after the index date. However, these three off-network visits all happened after the index date. Therefore, we believe that the off-network antibiotic therapy prior to the index date should be very minimal.

Another potential limitation is missing data. This is a common problem when conducting a study using information from the electronic medical record database. Since the Penn Data Store was not specifically designed for research purpose, some important variables may be unavailable. Additionally, completeness of data depends on patients' physicians and other related personnel. To ameliorate this problem, we carefully reviewed the infra-structure of Penn-data store and our study protocol to utilize all available information. We also performed a pilot study to validate the criteria for diagnosis of FQSEC UTI as previously mentioned.

The last potential limitation is generalizability. Our study population was drawn from a number of ambulatory practices within the UPHS; therefore it is possible that results of this study may not be applicable to populations in other settings or other geographic regions. To explore this issue,

we subsequently performed additional analysis on different groups of the population including 1) all subjects with FQSEC-bacteriuria (either true infection or colonization) and 2) both female and male subjects with non-recurrent FQSEC-UTIs. Independent risk factors identified in the bacteriuria model (902 cases and 5,483 controls) included Asian race [95%CI: 2.33; 1.41-3.81; p=0.001], race rather than black and white [95%CI: 1.65; 1.15-2.37; p=0.007] and underlying heart failure [95%CI: 2.58; 1.67-3.98; p<0.001]. Results from the both sex model (213 cases and 2,233 controls) revealed that Asian race [95%CI: 3.07; 1.48-6.37; p=0.003], underlying heart failure [95%CI: 2.21; 1.35-3.62; p=0.002] and previous exposure to nitrofurantoin [95%CI: 8.42; 1.86-38.11; p=0.006] were independently associated with high MIC-FQSEC UTIs. Although results from these three models (non-recurrent FQSEC-UTIs in female, asymptomatic FQSEC bacteriuria and non-recurrent FQSEC-UTIs in both sex) are similar, these results may be different in other clinical settings such as nosocomial UTIs, sicker populations, etc.

In conclusion, our study was the largest study specifically designed to identify risk factors for high MIC-FQSEC UTI. Our study revealed three independent risk factors including Asian race, underlying renal disease and previous exposure to nitrofurantoin. Data from chart-review confirmed that previous exposure to nitrofurantoin was a likely a proxy for prior UTI episodes that did not meet our study definitions for UTIs. Further studies are needed to explore the association between Asian race and high MIC-FQSEC UTIs.

4. TABLES

Table 3. Definition of urinary tract infections (UTIs)

To be diagnosed of UTI, the eligible subjects must meet **both criterion 1 and criterion 2.**	
Criterion 1	**Patient has a positive urine culture** $\geq 10^5$cfu/ml, with no more than two species of microorganisms
Criterion 2	**At least one of the following** a) ICD -9 code of signs and symptoms of UTIs b) Dipstick test positive for leukocyte esterase and/or nitrate c) Pyuria ($\geq$10 white blood cells (wbc) /mm^3 or $\geq$ 3 wbc /high power field of unspun urine) d) Physician diagnosis of a urinary tract infection (ICD-9 code) ▪ 599.0 Urinary tract infection, site not specified ▪ 590.x Infection of kidney ▪ 595.0 Acute cystitis ▪ 597.x Urethritis, not sexually transmitted diseases

Table 4. Sources for data collection

Data	Microbiology lab database	Penn Data Store
Study eligibility	✓	✓
Microbiological results and susceptibility profile	✓	
Clinic site and services		✓
Baseline demographic data		✓
Co-morbidities		✓
Previous hospitalization		✓
Medications used		✓
Treatment outcomes (for the cohort study)		✓

Table 5. Sample size calculation

Susceptibility	No. of isolates in 2010	Estimated number of isolates during the 3-year study period	Expected samples size during the 3-year study period
Low MIC- FQSEC Isolates	2684	8000	5600
High MIC-FQSEC Isolates	273	800	560

Table 6. Power calculation for the expected sample size

Prevalence of exposure in the control group	Prevalence of UTIs = 70% Among the source population (560 cases:5600 controls)		
	OR = 1.25	OR = 1.5	OR = 2.0
.10	0.36	0.82	0.99
.20	0.54	0.97	0.99
.30	0.65	0.99	0.99
.40	0.70	0.99	0.99
.50	0.71	0.99	0.99

Table 7. Power calculation for the exact sample size

Prevalence of exposure in the control group	Sample size = 2,001 subjects (165 cases and 1,836 controls)		
	OR = 1.5	OR = 2.0	OR = 3.0
.10	0.36	0.82	0.99
.20	0.55	0.97	0.99
.30	0.64	0.98	0.99
.40	0.68	0.99	0.99
.50	0.67	0.99	0.99

Table 8. Distribution of FQ susceptibility among subjects with *E. coli* bacteriuria, non-recurrent *E. coli* UTI episodes and female subjects with non-recurrent *E. coli* UTI

Susceptibility	*E. coli* bacteriuria	Non-recurrent episode of *E. coli* UTI	Adult Female with a non-recurrent episode of *E. coli* UTI
All	11,287 (100.0%)	3,418 (100%)	2,669 (100%)
1. FQSEC	8,461 (75.0%)	2,464 (72.1%)	2,001 (74.9%)
Low MIC	7,967 (68.8%)	2,252 (65.9%)	1,836 (68.8%)
High MIC	740 (6.4%)	212 (6.2%)	165 (6.1%)
2. FQIEC	49 (0.4%)	12 (0.4%)	11 (0.4%)
3. FQREC	2,777 (24.6%)	942 (27.6%)	658 (24.7%)

Table 9. Demographics, diagnosis criteria for UTI and comorbidity among the cases and controls.

Variables	High MIC (N=165)		Low MIC (N=1,836)		Unadjusted Odds Ratio (95% CI)	p-value
	N	%	N	%		
Demographics						
Age (Mean+/-SD)	56.91±22.57		57.34±21.98		0.99 [0.99-1.00]	0.81
Year of enrollment						0.51
▪ 2008	49	29.9	487	26.5	Ref	
▪ 2009	62	37.8	750	40.9	0.96 [0.68-1.36]	
▪ 2010	36	22	450	24.5	0.92 [0.62-1.36]	
▪ 2011	17	10.4	149	8.1	1.28 [0.76-2.15]	
Race						
▪ White	64	39.0	766	41.7	Ref	0.02
▪ Black	77	47.0	931	50.8	1.00 [0.71-1.41]	
▪ Asian	8	4.9	34	1.9	2.82 [1.25-6.34]	
▪ Other/Unknown	15	9.1	105	5.7	1.71 [0.94-3.11]	
Diagnosis Criteria for UTI						
Diagnosis by ICD-9	123	75	1250	68.0	1.41 [0.97-2.08]	0.07
▪ Sign and Symptom of UTI	5	3	61	3.3	0.92 [0.28-2.30]	0.85
▪ Pyuria	0	0	0	0	-	-
▪ Diagnosis code of UTI	123	75	1227	66.9	1.48 [1.02-2.19]	0.03
▪ UTI in pregnancy	36	22	400	21.8	1.01 [0.67-1.50]	0.95
Diagnosis by laboratory results	84	51.2	1016	55.3	0.85 [0.61-1.19]	0.32
▪ Microscopic pyuria	83	50.6	1003	54.6	0.85 [0.61-1.19]	0.32
▪ Positive urine leukocyte esterase test	61	37.2	746	40.6	0.87 [0.61-1.22]	0.39
▪ Positive urine nitrite	35	21.3	508	27.7	0.71 [0.47-1.05]	0.08
Co-morbidity						
▪ Charlson index (mean+/-SD)	0.45	0.79	0.4	0.72		0.35
▪ Having at least one	45	27.4	476	25.9	1.08 [0.74-1.56]	0.67

Charlson conditions						
▪ Acute Myocardial Infarction	7	4.3	45	2.5	1.77 [0.66-4.05]	0.16
▪ Congestive Heart Failure	16	9.8	78	4.3	2.44 [1.29-4.34]	0.001
▪ Peripheral Vascular Disease	4	2.4	48	2.6	0.98 [0.24-2.59]	0.57*
▪ Cerebrovascular disease	12	7.3	131	7.1	1.03 [0.51-1.91]	0.93
▪ Dementia	1	0.6	8	0.4	1.40 [0.03-10.56]	0.54*
▪ COPD	6	3.7	86	4.7	0.77 [0.27-1.79]	0.55
▪ Rheumatoid disease	2	1.2	12	0.7	1.88 [0.20-8.54]	0.32*
▪ Peptic Ulcer	2	1.2	10	0.5	2.25 [0.24-10.70]	0.26*
▪ Mild Liver Disease	1	0.6	13	0.7	0.86 [0.02-5.80]	0.99*
▪ Moderate/Severe Liver disease	0	0	6	0.3	-	0.99*
▪ All diabetes	5	3.0	36	2.0	1.57 [0.47-4.10]	0.38
▪ Diabetes without complication	2	1.2	23	1.3	0.97 [0.11-4.00]	0.66*
▪ Diabetes with complication	4	2.4	17	0.9	2.68 [0.65-8.33]	0.09*
▪ Hemiplegia or Paraplegia	2	1.2	13	0.7	1.73 [0.19-7.75]	0.35*
▪ Renal disease	12	7.3	68	3.7	2.05 [1.00-3.93]	0.02
▪ Cancer	8	4.9	134	7.3	0.65 {0.27-1.35]	0.25
▪ Metastatic cancer	4	2.4	37	2	1.22 [0.31-3.45]	0.57*
▪ AIDS	0	0	0	0	-	-

Note: * p-value from the non-parametric test

Table 10. Recent drug exposure within 90 days prior to the index date

Medication	High MIC (N=164)		Low MIC (N=1,836)		Unadjusted Odds Ratio (95% CI)	p-value
	N	%	N	%		
▪ All antibiotics	7	3.7	44	2.3	1.80 [0.67-4.12]	0.15
▪ Bactrim	1	0.6	5	0.3	2.25 [0.05-20.24]	0.40*
▪ Clindamycin	0	0	3	0.2	-	0.99*
▪ Linezolid	1	0.6	0	0	-	0.99*
▪ Metronidazole	0	0	6	0.3	-	0.99*
▪ Nitrofurantoin	2	1.2	4	0.2	5.65 [0.51-39.73]	0.08*
▪ Vancomycin	0	0	1	0.1	-	0.99*
▪ Cephalosporins	0	0	5	0.3	-	0.99*
▪ Fluoroquinolones	0	0	5	0.3	-	0.99*
▪ Aminoglycoside	0	0	0	0	-	-
▪ Penicillin	2	1.2	10	0.5	2.25 [0.24-10.70]	0.26*
▪ Beta-lactams	2	1.2	15	0.8	1.50 [0.16-6.53]	0.64
▪ Macrolide	0	0	2	0.1	-	0.99*
▪ Proton pump inhibitors (D0 to D90)	3	1.8	56	3.1	0.59 [0.11-1.86]	0.48
▪ H2blocker (D0 to D90)	3	1.8	16	0.8	2.12 [0.39-7.51]	0.20

Note: * p-value from the non-parametric test

Table 11. Independent risk factors for High MIC-FQSEC UTI (multivariable analysis)

High MIC-FQSEC UTI	Unadjusted OR [95% CI]	Adjusted OR [95% CI]	P-value
Race			0.02
▪ White	Ref	Ref	
▪ Black	1.00 [0.71-1.41]	0.99 [0.70-1.39]	
▪ Asian	2.82 [1.25-6.34]	2.92 [1.29-6.58]	
▪ Others	1.71 [0.94-3.11]	1.72 [0.94-3.15]	
Renal diseases	2.05 [0.99-3.93]	2.18 [1.15-4.14]	0.02
Previous exposure to Nitrofurantoin	5.65 [0.51-39.73]	8.86 [1.95-40.29]	0.005

PROJECT 2: CLINICAL IMPACT OF AMBULATORY URINARY TRACT INFECTIONS CAUSED BY HIGH MIC- FLUOROQUINOLONE SUSCEPTIBLE E. COLI IN WOMEN

1. METHODS

This study was nested within the case-control study (project-1). We conducted a retrospective cohort study to compare treatment response to FQ therapy between subjects with low MIC-FQSEC UTI and subjects with high MIC-FQSEC UTI. The study was approved by the Penn Institutional Review Board.

1.1 Study setting

Our study population was a subset of the case-control (the project-1) population. Same as the case-control study, the study was conducted at outpatient services within University of Pennsylvania Health System (UPHS), Philadelphia during May 1, 2008 – April 30, 2011.

1.2 Study subjects

Eligible subjects were those from the case-control study who received any FQ antibiotics as the first antibiotic regimen for UTI within 72 hours before or after an index urine culture was obtained. Subjects who did not receive any antibiotics within this capture period or received non-FQ antibiotic as the first course of antibiotic were excluded.

All subjects with high MIC-FQSEC UTI were enrolled into the exposed group while all subjects with low-MIC FQSEC UTI were enrolled into the unexposed group.

1.3 Study definitions and outcomes of interest

1) **Susceptibility to FQ** (as previously mentioned in the project-1)
2) **Index date:** The date that the first course of FQ therapy was initiated.
3) **Short-term treatment failure:** defined as any evidence of treatment failure documented between Day4 to Day14 after the index date.
4) **Long-term treatment failure:** defined as any evidence of treatment failure documented between Day15 to Week10 after the index date.

5) **Evidence of treatment failure:** A given subjects was documented as having treatment failure if at least one of the following criteria were true within the captured period.

- The second course of antibiotic therapy for UTI was prescribed
- There was any evidence of persistent or recurrent *E. coli* bacteriuria (At least 10^3 CFU/ml of *E. coli)*

1.4 Data collection

1) **Data source:** In addition to retrieving data from the HUP clinical microbiology database and the Penn Data Store, we additionally performed chart-review on the Epic and Medview systems to capture all possible events of treatment failure.
2) **Data to be collected**: All variables previously collected in the case-control study were also collected and considered potential confounders and effect modifiers in this study.

   Data on age, race, clinic site and service (e.g., medicine, surgery), designated primary care provider, previous hospitalization, comorbid conditions, previous and current medications used and microbiological results were obtained via the Penn data store. Microbiological data were obtained via the HUP clinical microbiology database. Treatment outcomes were obtained by performing chart-review.

   The presence of comorbid conditions was evaluated by applying the Enhanced ICD-9-CM coding algorithms to ICD-9 codes.[69,70] The comorbid conditions included myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular diseases, dementia, chronic obstructive pulmonary disease (COPD), liver disease, diabetes mellitus (with or without complications), chronic renal disease, malignancy and HIV infection.

Outpatient and inpatient medications used within 90 days prior and after the index date were evaluated. We recorded data of the following medications including antibiotics, steroid and other immunosuppressive agents.

Urine culture results within 90 days prior and after the index date were also obtained. Obtained data included the date of obtaining specimen, the infecting organism, the number of isolated colony forming unit, the susceptibility profile as well as the minimal inhibitory concentration (MIC).

3) **Data management:** This study used the same data management system as in the case-control study.

1.5 Statistical analysis

1) Introduction

Outcomes of interest included short-term treatment failure and long-term treatment failure while the exposure of interest was high MIC-FQ- susceptibility. Short-term and long-term outcomes were separately analyzed. The primary aim of this study was to determine the association between the high MIC-FQ susceptibility and treatment outcomes among subjects with ambulatory FQSEC UTI who received FQ therapy. Specific treatment outcomes evaluated included:

1) Composite treatment failure: defined as presence of at least 1 criterion of treatment failure (presence or absence)

2) Specific criteria for treatment failure as a dummy variable

- Requirement of second course of antibiotic therapy,
- Recurrent or persistent bacteriuria

For all calculations, a two-tailed P value of <0.05 was considered statistically significant. All calculations were performed using the STATA version 12.0 (Stata Corp, College Station TX).

2) Descriptive analysis

We described the data distribution of each collected variable and then characterized the exposed and unexposed groups by all characteristics including demographic variables, clinic site and service, comorbid conditions, previous medications used 3 months prior to the enrollment, type and duration of FQ therapy and treatment outcomes. Categorical variables were summarized by frequency while continuous variables were summarized by the mean, median, standard deviation, and range as appropriate.

3) Bivariable analysis

Characteristics and outcomes of the exposed group and the unexposed group were compared. Bivariable analysis was subsequently performed to determine the unadjusted association between the high MIC-FQ susceptibility and all variables. Furthermore, we also compared characteristics between subjects who experienced treatment failure and subjects who did not experience treatment failure to determine the unadjusted association between the treatment failure and all variables.

Categorical variables were compared using the chi-square or Fisher's exact test while continuous variables were compared using the student's t or Mann-Whitney U test, depending on the sample distribution. An odds ratio (OR) and its confidence interval (CI) were then reported.

4) Stratified analysis

Stratified analysis by Mantel-Haenszel test was subsequently performed to evaluate the effects of each variable of interest as a possible confounder as well as a possible interaction. The stratifying variables included year of enrollment, previous hospitalization, clinic site and service, age group, presence of comorbid conditions, type of FQ therapy (e.g. norfloxacin, ciprofloxacin, levofloxacin, etc) and duration of FQ therapy (≤ 3 days vs. >3 days). We determined whether these factors alter the association between exposure and the outcomes. The presence of confounding was documented if the difference between the crude effect estimate and the summary effect estimate was more than 15%.

Effect modification was assumed if the test for heterogeneity between the OR for different strata reaches statistical significance (P<0.05).

5) Multivariable analysis

Multivariable analysis was subsequently performed by using a multiple logistic regression model. Building of the multivariable model started with inclusion of our primary exposure variable (high MIC-FQ susceptibility). Other variables were considered for inclusion in a multivariable model if their bivariable p-value < 0.20 or they were found to be either confounder or interaction on stratified analysis. Forward-backward stepwise approach was used for selection of variables in the final model regardless of their p values. Finally, we also investigated the possible presence of interaction between variables.

6) Secondary analysis

a. Sensitivity analysis of MIC cut-off value

Because there is no standard MIC value to distinguish the low MIC-FQSEC from the high MIC FQSEC, we additionally performed the sensitivity analysis by changing the MIC-cutoff value for determining high vs. low MIC-FQ susceptibility. In this sensitivity analysis, an isolate with the MIC to levofloxacin ≤ 0.25 was considered low MIC-FQSEC while and isolate with the MIC to levofloxacin >0.25 but <4 was considered high MIC-FQSEC. The analysis methods used in this secondary analysis were exactly the same as those previously mentioned in the primary analysis.

b. Sensitivity analysis on outcome misclassification

Given the fact that we may underestimate the number of treatment failures, therefore the effect of high MIC-FQ susceptibility may be wrongly estimated. To explore this issue, we performed the sensitivity analysis on both patterns (differential and non-differential) and various degree of misclassification.

7) Sample size and power calculation

Only subjects from the case-control study who received FQ therapy were eligible for this study. Our expected prevalence of FQ therapy as the first antibiotic regimen was 80%

during May 2008-October 2010 (before the change in the IDSA guideline) and 20% during November 2010-April 2011 (after the change in the IDSA guideline). Given these numbers, we anticipated to enroll approximately 390 subjects in the exposed group and 3900 subjects in the unexposed group.

A previous clinical study reported that the long-term treatment failure in FQSEC-UTI patients who received a short course FQ therapy was 5%.[56] Therefore, we expected that the short-term treatment failure in our study population must be > 5%. Power calculations were performed by assuming that the prevalence of short-term treatment and long-term treatment failure among the unexposed group were 1% and 5%, respectively. Therefore, our study would have 99% and 95% power to detect at least 5% difference in the short-term and the long-term treatment failure, respectively. Detail of power calculation is shown in table 12.

Unfortunately, we were able enroll only 275 subjects into our cohort study (29 subjects into the exposed group and 246 subjects into the unexposed group). Based on this number, we still had more than 80% power to detect a difference in treatment response of 20% or more and the baseline treatment response is between 1-5% as shown in table 13. Since we believe that clinically significant difference in treatment failure is 20% or more, our study did have enough power to detect the clinically significant difference.

2. RESULTS

During a 3-year study period, a total of 279 eligible subjects were identified through the Penn Data Store (248 subjects in the low MIC group and 31 subjects in the high MIC group). Of these 279 eligible subjects, only 275 subjects had available medical records for review. Therefore, we finally enrolled 246 subjects into the low MIC group and 29 subjects into the high MIC group.

Median age (range) of the low MIC group and the high MIC group were 55 (18-99) years and 64 (18-89) years, respectively. Baseline characteristics between these two groups did not show

statistically significant differences in the bivariable analysis. Baseline characteristics between the high MIC vs. low MIC group are shown in table 14.

Two of the 246 subjects in the low MIC group and two of the 29 subjects in the high MIC group experienced short-term treatment failure (0.8% vs. 6.9%, p=0.06). Of these four subjects with short-term treatment failure, all of them had persistent signs or symptoms of UTIs and subsequently required the second course of antibiotic therapy. However, only one from four had a repeated urine culture, which later grew no pathogen. No long-term treatment failure was detected among our study population. Detail of treatment failure or each subject is shown in the table 15. Risk difference and risk ratio for short-term treatment failure were 0.06 [-0.03-0.15; exact-p=0.06] and 8.48 [1.24-57.97; exact-p=0.06], respectively.

Baseline characteristics of subjects who experienced (n=4) and who did not experience short-term treatment failure (n=271) were also compared. In the bivariable analysis, we found that short-term treatment failure was significantly associated with Asian race (p=0.04) and with underlying cerebrovascular disease (p=0.01). Additionally, having cardiovascular disease was slightly more prevalent among subjects who experienced short-term treatment failure, but this finding did not meet statistical significance. Table 16 shows baseline characteristics among subjects who experienced and who did not experience short-term treatment failure.

To explore confounding and interaction, we performed additional analysis including stratified analysis, building a multivariable model by including several potential confounders with and without interaction terms as well as building a propensity score-adjusted model. Results of these additional analyses are shown in table 17.

In the stratified analysis of subjects with and without at least one underlying disease, the crude RR [8.48; 95% CI = 1.24-57.97] was significantly different from the Mantel-Haenzel (MH)-combined RR [7.79; 95%CI = 1.16-52.39] with the MH p-value of 0.02. However, the difference of the crude RR and the summary RR was only 8%, and did not reach our pre-specified value (>15%) for determining significant confounding.

The stratified analysis on the underlying cerebrovascular disease (CVD) was also performed. The MH-combined RR of the underlying CVD [7.12; 95% CI=1.20-42.10] was also significantly different from the crude RR with the MH p-value of 0.04. The difference between the crude vs. summary RR of this variable was 16%, which reached the pre-specified value for the significant confounder.

Multivariable analysis was also performed to explore the effect of potential confounders and effect modifiers. By adding the variable of underlying CVD into the multivariable model, the odds ratio (OR) of high MIC-FQ susceptibility increased from 9.04 [95% CI=1.22-66.77; p=0.03] to 9.73 [95% CI=1.11-85.16; p=0.04]. However, adding the variable of having at least one underlying disease into the final model reduced the OR of the high MIC-FQ susceptibility to 8.53 [95% CI=1.14-63.96; p=0.04]. The model with an interaction term between the high MIC-FQ susceptibility and the variable of underlying CVD did not reach statistical significance. The model with an interaction term between the high MIC-FQ susceptibility and the variable of having at least one underlying disease did not convert. We did not include both potential confounders into the same model because they were collinear.

In addition to the stratified analysis and the multivariable analysis, we also built a propensity score model to predict the probability of being infected with the high MIC-FQSEC strain or the low MIC FQSEC strain. Due to a very low number of events in each underlying category, we failed to build a good predicting model. Therefore, it was impossible to reliably perform the propensity-score-adjusted analysis.

Since there is no standard MIC value to distinguish low MIC-FQSEC from high MIC- FQSEC, we additionally performed the sensitivity analysis by changing the levofloxacin MIC cut-off value for low MIC-FQ susceptibility from ≤0.12 to ≤ 0.25 mcg/mL. By using the new cut-off, a total of 252 subjects were classified into the low MIC group and 23 subjects were classified into the high MIC group. Two from 252 subjects in the low MIC group and two from 23 subjects in the high MIC-group experienced short-term treatment failure (0.8% vs. 8.7%, p=0.04). Risk difference and

risk ratio for short-term treatment failure between two groups were 0.08 [-0.40 -0.19; p=0.002] and 10.96 [1.62-74.21; p=0.002], respectively.

To explore the impact of misclassification, we performed sensitivity analysis on the various degree of differential and non-differential misclassification of outcomes. Table 18 shows the true effect estimates if non-differential misclassification or differential misclassification exists. In all hypothesized situations, the observed effect estimates were underestimated. Therefore, it is unlikely that the impact of high MIC-FQ susceptibility will go to the null.

3. DISCUSSION

Our study revealed that subjects with high MIC-FQSEC-UTIs were approximately 8 times more likely to experience treatment failure when received FQ therapy when comparing to those with low MIC FQSEC-UTIs (unadjusted RR=8.48 [1.24-57.97; exact-p=0.06]). Additionally, the variable of underlying CVD was documented as the significant confounder in our stratified analysis.

Results from the multivariable analysis revealed that the adjusted OR of the high MIC variable was comparable to the unadjusted OR. By adjusting with the variable of underlying CVD, the adjusted OR increased from 9.04 [95% CI=1.22-66.77; p=0.03] to 9.73 [95% CI=1.11-85.16; p=0.04]. Including the variable of having at least one underlying disease into the final model reduced the OR of the high MIC-FQ susceptibility to 8.53 [95% CI=1.14-63.96; p=0.04]. There was no evidence of interaction between these variables.

To support our significant findings, we also performed the sensitivity analysis of the MIC cut-off value as well as the sensitivity analysis of degree of misclassification. Results from both sensitivity analyses confirmed that the negative impact of the high MIC-FQ susceptibility is very unlikely to go to the null.

We believe that our study is superior to other previous studies in several aspects. First, our study definition to identify ambulatory UTIs has shown promising discrimination ability in our validation study. For this reason, only subjects with the real ambulatory UTIs were enrolled into

our study. Although the negative impact of high MIC-FQ susceptibility on treatment response to FQ therapy has been previously documented in infections caused by *Salmonella enterica* serovar Typhi (S. typhi),[59,60] our study was the first study exploring the impact of high MIC-FQ susceptibility on UTIs caused by *E. coli* pathogen. Furthermore, we exclusively focused only on ambulatory UTIs among women because the treatment response might vary across sites of infection and clinical settings.

Our study had several potential limitations. Since the subjects with high MIC-FQSEC-UTI may be sicker than subjects with low MIC-FQSEC UTI, this may result in a higher rate of treatment failure among the high MIC group. Therefore, we performed stratified analysis as well as multivariable analysis to explore this issue.

A major source of information bias in a cohort study is misclassification of the outcome. Although we used the specifically designed criteria to detect treatment failure, it is still possible that we may overlook some failure events. Since subjects who experience treatment failure may seek a second opinion at another medical provider, treatment failure could be underestimated. To address this issue, we also performed chart-review to identify documented off-network visit and treatment failure. Of these 275 study subjects, there was only one documented off-network visit occurred within the first 3 months after the index date. This off-network visit occurred in the low MIC group (0.4%, 1/275) and it was not correlated to the UTI episode. Therefore, information bias due to off-network visit should be very minimal. In addition to chart-review, we performed the sensitivity analysis to identify the true effect estimates in case non-differential or differential misclassification does exist. The sensitivity analysis revealed that the true effect estimates get bigger in the presence of either non-differential or differential misclassification.

Another potential limitation is generalizability. This study primarily focused on female subjects with non-recurrent ambulatory FQSEC-UTIs. Therefore, the results of this study would not be applicable to recurrent UTIs, UTIs caused by other pathogens, other sites of infection as well as UTIs in the non-ambulatory setting.

The last important limitation is our unexpectedly small sample size. Based on this small sample size, we do not have a sufficient power to identify the difference if the baseline treatment failure is higher than 5% and the difference in treatment failure is less than 20%. Nevertheless, we believe that it is not clinically meaningful if the difference in treatment failure is less than 20%.

In conclusion, our study was the first study demonstrating the negative impact of the high MIC-FQ susceptibility on the treatment response among female subjects with ambulatory UTIs caused by *E. coli* who received FQ therapy. We believe that the negative impact of high MIC-FQSEC may be more intensified in more serious clinical situations such as nosocomial UTIs, complicated intra-abdominal infections or bacteremia. Future studies in other clinical settings should be conducted to fill the gap of knowledge.

4. TABLES

Table 12. Power calculation for the expected sample size

Treatment failure in the low vs. high MIC group	Difference	Power if N= 390/3900
1% vs. 2%	1%	0.38
1% vs. 3%	2%	0.80
1% vs. 4%	3%	0.95
1% vs. 5%	4%	0.99
1% vs. 6%	5%	1.00
5% vs. 6%	1%	0.13
5% vs. 7%	2%	0.37
5% vs. 8%	3%	0.65
5% vs. 9%	4%	0.85
5% vs. 10%	5%	0.95

Table 13. Power calculation for the real sample size

Treatment failure in the low vs. high MIC group	Difference	Power if N= 29/246
1% vs. 16%	15%	0.85
2% vs. 17%	15%	0.80
3% vs. 18%	15%	0.76
4% vs. 19%	15%	0.72
5% vs. 20%	15%	0.70
1% vs. 21%	20%	0.93
2% vs. 22%	20%	0.91
3% vs. 23%	20%	0.89
4% vs. 24%	20%	0.87
5% vs. 25%	20%	0.84

Table 14. Baseline characteristics of the low MIC group and the high MIC group

Variables	Low MIC (N=246)		High MIC (N=29)		p-value
	N	%	N	%	
Baseline Characteristics					
Age (Mean+/-SD)	55.62+/-22.69		59.51+/-20.75		0.38
Age (Median, range)	55 (18-99)		64 (18-89)		0.35
Year of enrollment					
2008	82	33.3	9	31.0	0.8
2009	131	53.3	15	51.7	
2010	33	13.4	17.2	16.1	
Race					
White	89	36.2	15	51.7	0.14*
Black	141	57.3	11	37.9	
Asian	4	1.6	1	3.5	
Other/unknown	12	4.9	2	6.9	
Co-morbidity					
Charlson index (mean +/-SD)	0.33+/-0.66		0.45+/-0.78		0.37
Charlson index (median, range)	0 (0-2)		0 (0-2)		0.44*
Having at least one Charlson conditions	54	22.0	8	27.6	0.49
Acute myocardial infarction	4	1.6	1	3.5	0.43*
Congestive heart failure	8	3.3	3	10.3	0.10*
Peripheral vascular disease	6	2.4	0	0.0	0.99*
Cerebrovascular disease	12	4.9	2	6.9	0.65*
Dementia	3	1.2	0	0.0	0.99*
COPD	14	5.7	1	3.5	0.99*
Rheumatoid disease	1	0.4	0	0.0	0.99*
Peptic ulcer	1	0.4	0	0.0	0.99*
Mild liver disease	0	0.0	1	3.5	0.11*
Moderate/severe liver disease	0	0.0	0	0.0	…
Diabetes	3	1.2	1	3.5	0.36*

Diabetes without complication	2	0.8	0	0.0	0.99*
Diabetes with complication	1	0.4	1	3.5	0.20*
Hemiplegia or paraplegia	1	0.4	0	0.0	0.99*
Renal disease	6	2.4	2	6.9	0.20*
Cancer	12	4.9	1	3.5	0.99*
Metastatic cancer	7	2.9	0	0.0	0.99*
AIDS	0	0.0	0	0.0	…

Note: * p-value from the non-parametric test

Table 15. Detail of treatment failure of each subjects

Findings	Low MIC		High MIC	
	Case 1	**Case 3**	**Case 2**	**Case 4**
Type of UTIs	Cystitis	Cystitis	Acute pyelonephritis	Cystitis
Detail of the first antibiotic prescription	Ciprofloxacin 500 mg PO bid for 5 days	Levofloxacin 500 mg PO for 7 days	Levofloxacin 500 mg IV od	Ciprofloxacin 500 mg PO bid for 7 days
Date of documented treatment failure (after the index date)	Day-12	Day-8	Day-4	Day-9
Evidence of treatment failure	▪ Dysuria persisted on Day-12 ▪ Levofloxacin 500 mg PO od for 5 days was prescribed on Day-12	▪ Dysuria persisted on Day-8 ▪ Nitrofurantoin 100 mg PO bid for 7 days was prescribed on Day-8	▪ Persistent fever on Day-4 ▪ Levofloxacin was discontinued and cefipime was prescribed	▪ Dysuria persisted on Day- 9 ▪ Ciprofloxacin 500 mg bid for 7 days was represcribed on Day-9
Repeated urine culture	Yes (on Day-12)	No	No	No
Results of repeated urine culture	Normal flora	-	-	-

Table 16. Baseline characteristics among subjects who experienced and who did not experience short-term treatment

Variables	No Failure (n=271)		Failure (n=4)		p-value
	N	%	N	%	
Age (Mean +/-SD)	55.89 +/-22.51		65.75 +/- 21.31		0.38
Age (Median, range)	57 (18-99)		65.5 (45-87)		0.37*
Year of enrollment					
2008	89	32.8	2	50.0	0.80*
2009	144	53.1	2	50.0	
2010	38	14.0	0	0.0	
Race					
White	101	37.3	3	75.0	0.04*
Black	152	56.1	0	0.0	
Asian	5	1.9	0	0.0	
Other/unknown	13	4.8	1	25.0	
Co-morbidity					
Charlson index (mean +/-SD)	0.34+/-0.67		0.75+/-0.96		0.23
Charlson index (median, range)	0 (0-2)		0 (0-2)		0.19*
Having at least one Charlson conditions	60	22.1	2	50.0	0.22*
Acute myocardial infarction	4	1.5	1	25.0	0.07*
Congestive heart failure	11	4.1	0	0.0	0.99*
Peripheral vascular disease	6	2.2	0	0.0	0.99*
Cerebrovascular disease	12	4.4	2	50.0	0.01*
Dementia	3	1.1	0	0	0.99*
COPD	15	5.5	0	0	0.99*
Rheumatoid disease	1	0.4	0	0	0.99*
Peptic ulcer	1	0.4	0	0	0.99*
Mild liver disease	1	0.4	0	0	0.99*
Moderate/severe liver disease	0	0	0	0	..
Diabetes	4	1.5	0	0	0.99*

Diabetes without complication	2	0.7	0	0	0.99*
Diabetes with complication	2	0.7	0	0	0.99*
Hemiplegia or paraplegia	1	0.4	0	0	0.99*
Renal disease	8	3.0	0	0	0.99*
Cancer	13	4.8	0	0	0.99*
Metastatic cancer	7	100.0	0	0	0.99*
AIDS	0	0	0	0	...

Note: * p-value from the non-parametric test

Table 17. Results from stratified analysis and multivariable analysis of factors associated with the short-term treatment failure

1. Stratified analysis	**Subgroup**	**RR [95%CI]**	**MH P-value**
1.1 Having at least one underlying diseases	Yes	6.75 [0.47-97.52]	0.02
	No	9.14 [0.59-140.88]	
	Crude	8.48 [1.24-57.97]	
	MH-Combined	7.79 [1.16-52.39]	
1.2 Having underlying of CVD*	Yes	6.00 [0.58-61.84]	0.04
	No	8.67 [0.56-134.63]	
	Crude	8.48 [1.24-57.97]	
	MH-Combined	7.12 [1.20-42.10]	
2. Model	**Variables in the model**	**OR [95%CI]**	**P-value**
2.1 Simple logistic regression model	High MIC	9.04 [1.22-66.77]	0.03
2.2 Simple logistic regression model	Having underlying CVD*	21.58 [2.80-166.60]	<0.001
2.3 Simple logistic regression model	Having at least one underlying diseases	3.52 [0.49-25.49]	<0.001
2.4 Multiple logistic regression model	High MIC	9.73 [1.11-85.16]	0.04
	Having underlying CVD*	23.03 [2.61-203.49]	0.005
2.5 Multiple logistic regression model	High MIC	8.53 [1.14-63.96]	0.04
	Having at least one underlying diseases	3.22 [0.43 -24.15]	0.26
2.4 Multiple logistic regression model	High MIC	8.96 [0.54-147.56]	0.13
	Having underlying CVD*	21.28 [1.24-361.46]	0.04
	Interaction term	1.23 [0.01-104.14]	0.93
2.6 Multiple logistic regression model	High MIC	...	Model did not convert
	Having at least one underlying diseases	...	
	Interaction term	...	

Note. *CVD stands for cerebrovascular disease

Table 18. True effect estimates if non-differential misclassification or differential misclassification exists

Pattern of Misclassification		High MIC (N=29)		Low MIC (N=246)		Risk Difference	Risk Ratio
		Fail	Not Fail	Fail	Not Fail		
Observed outcomes		2.0	27.0	2.0	244.0	0.06	8.48
Non-differential misclassification							
10%	10%	2.2	26.8	2.2	243.8	0.07	8.48
20%	20%	2.5	26.5	2.5	243.5	0.08	8.48
30%	30%	2.9	26.1	2.9	243.1	0.09	8.48
40%	40%	3.3	25.7	3.3	242.7	0.10	8.48
50%	50%	4.0	25.0	4.0	242.0	0.12	8.48
Differential misclassification							
20%	10%	2.5	26.5	2.2	243.8	0.08	9.64
30%	20%	2.9	26.1	2.5	243.5	0.09	9.84
40%	30%	3.3	25.7	2.9	243.1	0.10	9.65
50%	40%	4.0	25.0	3.3	242.7	0.12	10.28
10%	20%	2.2	26.8	2.5	243.5	0.07	7.46
20%	30%	2.5	26.5	2.9	243.1	0.07	7.31
30%	40%	2.9	26.1	3.3	242.7	0.09	7.45
40%	50%	3.3	25.7	4.0	242.0	0.10	7.00

Project 3: A comparison of the case-control study vs. the case-case-control study to investigate risk factors for antimicrobial resistance in simulated population

1. ABSTRACT

BACKGROUND: Nowadays, the case-control study is the most common study design used for exploring risk factors for antimicrobial resistance. The newer approach "case-case-control" study has been proposed to be a better alternative design to identify risk factors for antimicrobial resistance. Thus far, the conventional case-control (CC) approach and the novel case-case-control (CCC) approach have never been quantitatively contrasted and compared.

METHODS: A study investigating risk factors for infection caused by FQ resistant pathogen was used as a standard model in our simulation study. We evaluated both the CC approach and the CCC approach across 432 reasonable clinical situations. In each clinical situation, 500 simulated datasets were created by Monte Carlo simulation and subsequently used for conducting a case-control study. Effect estimates of previous FQ exposure on infection caused by FQR pathogen from both approaches were quantitatively compared.

RESULTS: Based on data from our study, the effect of prior fluoroquinolone (FQ) exposure (X) on the FQ-resistant infection identified by the CC approach is remarkably different from those identified by the CCC approach. The difference is more pronounced if the study was conducted in healthy population, with a lower colonization rate of 10% and no protective effect of FQ exposure on mechanism of harboring FQ-susceptible pathogen.

CONCLUSION: These findings support the results in previous literatures which concluded that the CC approach almost always overestimates the effect of previous antibiotic exposure. However, that the difference between the CC and the CCC approaches would be significant only when the protective effect of exposure on mechanism of harboring FQ-susceptible pathogen (A_1) does not exist. Furthermore, this difference is more pronounced in healthy population with a low colonization rate which a number of subjects who have FQ-susceptible and FQ-resistant colonization should be very low..

2. BACKGROUND

Emergence of antimicrobial resistance is considered a global health problem. Knowing risk factors for antimicrobial resistance is an essential step in reducing the spread of antibiotic-resistant pathogens. Currently, the case-control study is the most common study design used for exploring risk factors for antimicrobial resistance. The newer approach "case-case-control" study has been proposed to be a better alternative design to identify risk factors for antimicrobial resistance. Thus far, the conventional case-control (CC) approach and the novel case-case-control (CCC) approach have never been quantitatively contrasted and compared.

Mechanisms of infection[4,29,80]

To accurately explore risk factors for developing infection caused by resistant pathogens, it is necessary to understand mechanisms of infection. Two necessary steps are required in developing of infection include: 1) harboring a causative pathogen, and 2) being infected by that particular pathogen. A model of infection caused by fluoroquinolone-resistant (**FQR)** and fluoroquinolone-susceptible (**FQS)** bacteria are used as an example here. The mechanisms of infection and other associated factors are shown in figure 1.

An individual can harbor FQS bacteria via mechanism-1 while mechanism-2 and mechanism-3 are for harboring FQR bacteria. For the **mechanism-1**, an individual harbors only **FQS** bacteria. For the **mechanism-2,** an individual directly harbors **FQR** bacteria from either direct or indirect transmission. The **mechanism-3** starts when an individual harbors FQS bacteria, then these particular bacteria become resistant to FQ either by intrinsic mutation or acquisition of resistance genes from other pathogens. These bacteria may come from either exogenous source (e.g. droplet transmission of *Streptococcus pneumoniae*) or endogenous source (e.g. fecal colonization of *Enterobacteriacae* spp.). After harboring a causative pathogen, the next step for developing infection is being infected by these causative bacteria. An individual with FQR bacteria may later be infected by these FQR bacteria. It is possible that this individual may be free of infection caused by these bacteria his/her entire life. Similarly, an individual who harbored

FQS bacteria may or may not be infected by these FQS bacteria. However, it is impossible for an individual who does not harbor any bacteria to develop infection.

Conventional case-control study

A conventional case-control design has been widely accepted as a valid approach to explore risk factors for antimicrobial resistance. A conventional analysis approach for this design is comparing the resistant cases (patients with infection caused by a resistant pathogen) to the susceptible controls (patients with infection caused by a susceptible pathogen). When the resistant cases are compared with the susceptible controls, the effect estimate of a particular risk factor represents the likelihood of being infected by the resistant strains for the infected individual who has that given factor. In this study design, previous exposure to a particular antibiotic is almost always found to be a risk factor for resistance of that particular antibiotic. This is the results of a combination of two effects—the effect of antibiotic exposure in selecting for resistant pathogens (increasing the frequency of cases) and the protective effect of the exposure in reducing the frequency of controls. For example, receiving FQ therapy prior to the time of culture can inhibit growth of FQS pathogens. Thus, if this person develops a clinical infection (which usually arise from an organism colonizing the individual), they are less likely to be infected with FQS bacteria. This person is unlikely to develop infection caused by FQS because he/she is less likely to be colonized with FQS bacteria. Therefore, the effect of previous FQ therapy on the population risk of a FQR infection may be overestimated in the conventional case-control study. These limitations have been widely discussed over the past decade.[81-83]

Novel approach, "Case-case-control study"

Kaye et al. proposed a novel study design, "the case-case-control study" as an alternative approach to the standard case-control study. The goal of the new design is to draw more accurate conclusions on risk factors for resistant infection.[83] In addition to comparing the resistant cases with the uninfected controls, they suggested comparing the susceptible cases to the uninfected controls. When the resistant cases are compared with the uninfected controls, the combined risk factors for infection with the pathogen of interest in general, and infection caused

by the resistant strains specifically, are identified. When the susceptible cases are compared with the uninfected controls, the risk factors for developing infection caused by the pathogen of interest in general are identified. By comparing and contrasting the results from these two models qualitatively, the risk factors specifically associated with infection caused by resistant strain are potentially identified.

Relative Merits of each approach

Results from the CC approach directly address the question of "if a patient develops an infection, what are the risk factors for that infection being FQ resistant". As a predictive model, it might also assist clinicians in selecting antibiotic therapy before the full susceptibility profile of the pathogen is known. The CCC approach directly answers the question "what are the risk factors for FQR infection among the population at risk of the infection". This question may be of greater interest to healthcare epidemiologists to implement an effective program to prevent spreading of resistant organisms.

Although a number of epidemiology experts have widely explored the limitations of the CC approach[82-84], all of them only qualitatively compared the results from the CC approach to the CCC approach. To date, there has never been any studies that quantitatively compare and contrast the results from these two approaches.

This study was conducted to evaluate the CC vs. CCC approach in investigating risk factors for antimicrobial resistance which will help researchers select the best approach to identify risk factors for antimicrobial resistance. This valuable knowledge is not only useful for any physician to take care of their patients, but also to policy makers and researchers.

3. METHODS

A study investigating risk factors for infection caused by FQ-resistant pathogen was used as a standard model in our simulation study. We evaluated both the CC approach and the CCC approach across 432 reasonable clinical situations. In each clinical situation, 500 simulated datasets were created by Monte Carlo simulation and subsequently used for conducting a case-

control study. Effect estimates of previous FQ exposure on infection caused by FQR pathogen from both approaches were quantitatively compared.

3.1 Synthetic cohort population

Characteristics of our synthetic cohort population were pre-specified to mimic baseline characteristics of subjects from previous studies investigating risk factors for antimicrobial resistance.

1) **Structures of the synthetic cohort population**

Synthetic cohort population with the multinomial outcome (no infection (Y=0), infection caused by FQS bacteria (Y=1) and infection caused by FQR bacteria (Y=2)) and binary primary exposure (absence (X=0) or presence (X=1) of previous FQ exposure) was specifically generated for this study.

As mentioned above, development of infection requires two essential steps; harboring a causative pathogen and being infected by that particular pathogen. We created three binomial variables, A_1, A_2 and A_3 to represent the mechanism of harboring the causative pathogen 1, 2 and 3, respectively. It is possible that an individual may not harbor any pathogens at all (A_1=0, A_2=0, A_3=0). Also, the mechanism-2 and 3 may be presented in the same individual (A_2=1 and A_3=1). However, we did not allow the mechanism-1 to simultaneously present with the mechanism-2 or 3. Variable B represents the infection status of patients which was coded as no-infection (B=0) and having infection (B=1).

Two additional covariates were also created. We generated the covariate-1 (C_1) as a continuous variable to represent the subject's age (mean+/-SD) and the covariate-2 (C_2) as a binary variable to represent the absence (C_2=0) or presence (C_2=1) of underlying disease(s). Structure of the synthetic cohort population is shown in figure 2.

2) **Population characteristics and pre-specified variables**

The population size was set to 100,000 subjects while the sample size was set to 200 subjects per group. Since a study investigating antimicrobial resistance can be done either in healthy population or sick population, therefore we generated two separated sets of

population; 1) the healthy population and 2) the sick population. Characteristics of both population sets are shown in table 19.

Data from previous studies of ambulatory UTIs caused by *E. coli*[40,43,45] was used to pre-specify several key parameters including the prevalence of infection among the overall population, the prevalence of FQR infection among infected individual, the prevalence of prior FQ exposure among the overall population as well as population's characteristics.

3.2 Clinical situations

To identify all reasonable clinical situations, three important aspects need to be considered. The first aspect is the colonization rate, which usually varies across the causative pathogen. Second is the proportion of mechanism-2: mechanism-3 (A_2:A_3) which may be different across clinical situations. Lastly, the true pattern of association between X, B, A_1, A_2 and A_3 which is still uncertain, therefore all possible patterns of association should be evaluated. Details of these three important aspects are explained below.

1) **Colonization rate**

   Given that the colonization rate usually varies across the causative pathogen, the colonization rate was set to 10% and 100% as shown in table 20. The colonization rate of 100% is suitable for any pathogen which is considered a normal flora in human (i.e. enteric *E. coli*), while the colonization rate of 10% is more suitable for other non-local organisms.

2) **Proportion of mechanism-2:mechanism-3**

   The proportion of mechanism-2: mechanism-3 (A_2:A_3) may differ across clinical situations. For instance, the mechanism-2 is likely to be the main mechanism of harboring resistant pathogen in an outbreak of Methicillin-Resistant *Staphylococcus aureus* (MRSA) surgical site infections. On the other hand, the main mechanism of harboring resistant pathogen among patients with chronic obstructive lung disease who developed penicillin-resistant *Streptococcus pneumoniae* (PRSP) pneumonia should be the mechanism-3. For this reason, the proportion of mechanism-2: mechanism-3 was set to 10:90, 50:50 and 100:0 as shown in the table 20.

3) Patterns of association and base equation (BE)

Four base equations were used to determine the association between the primary exposure, the primary outcome as well as covariates. All base equations are shown below and the pre-specified values for their corresponding beta-coefficients are demonstrated in table 21.

Base equation-1: association between C_1 and C_2

BE-1: logit p (C_2) = $\beta_0 + \beta_1 C_1$

Base equation-2: association between C_1, C_2 and X

BE-2: logit p (X) = $\beta_0 + \beta_1 C_1 + \beta_2 C_2$

Base equation-3: association between A_n and X (n=1, 2, and 3)

BE-3: logit p (A_n) = $\beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 X$

Base equation-4: association between B and X

BE-4: logit p (B) = $\beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 X$

All variables (X, A_1, A_2, A_3 and B) were set to be positively associated with both covariates (C_1 and C_2) with a beta-coefficient of 0.69314718 [OR=2.0]. Given that the association between the prior FQ exposure (X) and the mechanism of harboring the FQS pathogen (A_1) can be either negative or null, therefore the possible beta coefficients were set to -0.69314718 [OR=0.5] and 0.00000001 [OR=1.0].

Prior FQ exposure (X) can have either positive effect or no effect, but not a negative effect, on both mechanisms of harboring the FQR pathogen (A_2 and A_3). Therefore, their possible beta coefficients were set to 0.00000001 [OR=1.0] and 0.69314718 [OR=2.0]. Based on the concept of selective pressure of antibiotic use, it is reasonable to believe that the prior FQ exposure (X) may have stronger impact on the intrinsic development of FQR pathogen (A_3) when comparing to the mechanism of harboring extrinsic FQR pathogen (A_2). Given this reason, the beta-coefficient of 1.0986123 [OR=3.0] was also added for an association between the mechanism-3 (A_3) and the prior FQ exposure (X). Moreover, the true association between the prior FQ exposure (X) and the probability of being infected (B)

is still uncertain. So, the beta-coefficient of X on B was set to -0.69314718 [OR=0.5], 0.00000001 [OR=1.0] and 0.69314718 [OR=2.0].

Given that there were two possible colonization rates, three possible proportions of mechanism-2: mechanism-3 and 36 possible patterns of association (2*2*3*3 = 36), a total number of reasonable situations was 216 (2*3*36). Since we generated two population groups (healthy and sick population), a total of 432 clinical situations were evaluated.

3.3 Monte Carlo Simulations

Monte Carlo simulation was performed by STATA version 12.1/SE. All random variables were generated by using the random number function in the STATA program. Normal and uniform random numbers were generated by the methods derived by Knuth[85]. Initial value of random-number was specified (set seed command) to ensure the reproducibility.

3.3.1 Creating the synthetic cohort population

The synthetic cohort population was created step-by-step by using the aforementioned base equations. Our simulation steps are described below.

1) The continuous C_1 was generated as a normally distributed variable with a mean+/-SD of 60+/-10.
2) By using the ***BE-1,*** the C_2 was generated as a binomial variable.
3) The binomial variable of X was then generated by using the ***BE-2***.
4) The ***BE-3*** was used to generate the binomial A_1, A_2 and A_3. Furthermore, the colonization rate and the proportion of A_2: A_3 had to be taken into account. Therefore, the total number of subjects with colonization must be equal to the total number of subjects with A_1=1 or A_2=1 or A_3=1. Also, the total number of subjects with A_2=1 or A_3=1 must be matched to our prespecified proportion.
5) The new A_4 variable was then generated by using information from the A_1, A_2 and A_3. (A_4=0 for no colonization or FQS colonization, A_4=1 for FQR colonization).
6) The binomial B was generated by using the ***BE-4.***

7) At the last step, we created four variables to represent the final outcome (Y) which is a composite outcome of A and B

- Y as a multinomial variable: no infection (Y=0), FQS infection (Y=1), FQR infection (Y=2)
- Y_1 as a binomial variable: FQS infection (Y_1=0), FQR infection (Y_1=1)
- Y_2 as a binomial variable: No infection (Y_2=0), FQR infection (Y_2=1)
- Y_3 as a binomial variable: No infection (Y_3=0), FQS infection (Y_3=1)

3.3.2 Analytic approach

For each clinical situation, we generated 500 datasets of synthetic cohort population for conducting a case-control study by the CC approach as well as the CCC approach. Effect estimates from each approach, each simulated data set and each clinical situation were separately recorded. Detail of analytic approach is explained below.

1) Conventional case-control (CC) approach

In this approach, we conducted a case-control study by building a model comparing 200 case subjects with FQR infection (Y_1=1) to 200 control subjects with FQR infection (Y_1=0). Cases and controls were randomly sampled from 100,000 subjects in the synthetic cohort population. Model building was performed using logistic regression. The effect estimate (Odd Ratio) of previous FQ exposure (X) on the outcome of interest (Y_1) was then recorded across various clinical situations.

Model-1: logit p (Y) $= \beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 X$

OR for X $= \exp(\beta_3)$

Where: **Y_1** is a binary variable representing FQS infection (Y_1=0) or FQR infection (Y_1=1).

C_1 is a continuous variable representing subject's age (mean+/-SD)

C_2 is a binary variable representing presence (C_2=1) or absence (C_2=0) of underlying disease

X is a binary variable representing presence (X=1) or absence (X=0) of previous FQ exposure.

β_0 is a constant term

β_1 is a beta-coefficient for C_1.

β_2 is a beta-coefficient for C_2.

β_3 is a beta-coefficient for X.

2) Case-case-control (CCC) approach

We conducted a case-control study by building two separated models; First (model-2): a model comparing 200 case subjects with FQR infection to 200 controls subjects without infection; Second (model-3): a model comparing 200 case subjects with FQS infection to 200 control subjects without infection. All study subjects were randomly sampled from 100,000 subjects in the synthetic cohort population. Model building was performed by using the same steps as the CC approach. The effect estimates (Odd Ratio) from the model-2 and the model-3 were separately recorded across the various situations.

Model-2: logit p (Y_2) = $\beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 X$

OR for X = exp (β_3)

Where: **Y_2** is a binary variable representing no infection (Y_2=0) or FQR infection (Y_2=1).

C_1 is a continuous variable representing subject's age (mean+/-SD)

C_2 is a binary variable representing presence (C_2=1) or absence (C_2=0) of underlying disease

X is a binary variable representing the presence (X=1) or absence (X=0) of previous FQ exposure.

β_0 is a constant term

β_1 is a beta-coefficient for C_1.

β_2 is a beta-coefficient for C_2.

β_3 is a beta-coefficient for X.

Model-3: logit p (Y_3) = $\beta_0 + \beta_1 C_1 + \beta_2 C_2 + \beta_3 X$

OR for X = exp (β_3)

Where: **Y_3** is a binary variable representing no infection (Y_3=0) or FQS infection (Y_3=1).

C_1 is a continuous variable representing subject's age (mean+/-SD)

C_2 is a binary variable representing presence (C_2=1) or absence (C_2=0) of underlying disease

X is a binary variable representing the presence (X=1) or absence (X=0) of previous FQ exposure.

β_0 is a constant term

β_1 is a beta-coefficient for C_1.

β_2 is a beta-coefficient for C_2.

β_3 is a beta-coefficient for X.

3) True effect of primary exposure (previous FQ exposure)

Similar to the CC approach and the CCC approach, we evaluated the true effect of prior FQ exposure (X) on the final outcome by building a multiple logistic regression model-1, -2 and -3. However, all subjects in the synthetic cohort population (n=100,000) were included into the analysis.

3.3.3 Parameters to be evaluated

After performing all analyses, we obtained both estimated OR and the true OR of X on other variables in across all possible clinical situations. Parameters to be reported are shown below.

1)Estimated OR from the CC approach and the CCC approach

The effect estimates of the prior FQ exposure (X) on outcomes of interest (Y_1, Y_2 or Y_3) are presented as the odds ratio and 95% confident interval.

2)True OR from the entire cohort population

The true effect of the prior FQ exposure (X) on outcomes of interest (Y_1, Y_2 or Y_3) as well as the probability of being infected (B) are presented as the odds ratio and 95% confident interval.

3)Model comparison

3.1) Percent bias (PB) and Percent deviation (PD)

PB and PD can be calculated by using the following formula.

$$PB = \frac{\text{estimated value - true value}}{\text{true value}} \times 100\%$$

$$PB = \frac{\text{model B-estimated value - model A-estimated value}}{\text{model A-estimated value}} \times 100\%$$

The PB represents the average tendency of the estimated value to be larger or smaller than its true value. A positive PB indicates overestimation bias while a negative PB indicates underestimation bias. The PD represents the average difference between two values. Therefore, a bigger value indicates a larger difference between two observed values.

3.2) Coverage probability (CP)

CP is the proportion of the time that the confidence interval of the estimated value contains the true value of interest. Ideally, the CP would be about 0.95 (since they are based on 95% confidence intervals)

.

4. RESULTS

To evaluate the CC and CCC approach, results from the following pairs of model were compared.

4.1 Estimated effect vs. True effect

An effect estimate of X on Y_1 from the case-control study and an effect estimate of X on Y_2 or Y_3 from the case-case-control study were compared to the true which was identified from the entire cohort population. This helps us to assess the degree of bias when conducting the case-control study across various clinical situations. Supplementary table 1 shows the estimated OR, the true

OR, the percent bias (PB) as well as the coverage probability (CP) when the estimated OR and the true OR were compared.

When we focused on the model-1 (FQR vs. FQS), the mean PB was 6.47 ± 3.79 and the median PB was 5.51 (range -0.75 - 22.73). The mean CP was 0.96 ± 0.01 and the median CP was 0.96 (range 0.93 - 0.99). The PB was higher than 20% in 5 clinical situations. However, the CP was higher than 80% in all clinical situations. Population characteristics and patterns of association of these 5 outliers are shown in the table 23. These outliers occurred in a study conducted among the healthy population, without the protective effect of X on A_1 (beta-coefficient = 0.0000001) and with small effect of X on B (beta-coefficient = 0.69314718).

When we focused on the model-2 (FQR vs. No infection), the mean PB was 5.58 ± 2.27 and the median PB was 5.39 (-0.2 - 11.7). Mean CP was 0.95 ± 0.01 and the median CP was 0.95 (0.92 - 0.98). There was no any clinical situation that the PB was higher than 20% or the CP was below 80%.

When we focused on the model-3 (FQS vs. No infection), the mean PB was 4.75 ± 2.23 and the median PB was 4.47 (0.56 - 12.1). Mean CP was 0.95 ± 0.01 and the range was 0.95 (0.93 - 0.97). There was no any clinical situation that the PB was higher than 20% or the CP was below 80%.

4.2 Comparison of the estimated effect from the model-1 vs. the model-2

An effect estimate of X on Y_1 from the case-control study (estimated OR_{Y1}) was compared to the effect estimate of X on Y_2 from the case-case-control study (estimated OR_{Y2}). This helps us to determine the clinical situations that the model-1 and the model-2 would provide the similar results.

Percent deviation (PD) and coverage probability (CP) between the estimated OR_{Y1} and the estimated OR_{Y2} are shown in supplementary table 2. The mean PD was 51.86 ± 80.00 and the median PD was 26.68 (-49.48 - 274.74). Figure 3 shows the percent deviation across all possible clinical situations. The CP was 0.56 ± 0.30 and the median CP was 0.53 (0.05 - 0.96).

Sensitivity analysis was subsequently performed to determine whether the PD and CP may vary by some specific factors. Figure 4 shows the sensitivity analysis on the population type (Healthy vs. Sick population). Figure 4.1 and 2.2 present the PD from the healthy population and the sick population, respectively. The x-axis represents the patterns of association. The PD was obviously higher among the healthy population when comparing to the sick population. Nineteen outliers (PD>300%) are located at the left upper corner of the figure 4.1, which confirms that the pattern of association has significant impact on the PD. Population characteristics and patterns of association of these 19 outliers are shown in the table 24. These high bias patterns occurred in situations that there is no protective effect of X on A_1 (beta-coefficient =0.0000001) but there is small effect of X on B (beta-coefficient =0.69314718).

Figure 5 shows the sensitivity analysis on the colonization rate (10% vs. 100). Figure 5.1 and 5.2 reveal the PD from the population with a colonization rate of 10% and 100%, respectively. The PD was slightly higher among the clinical situation with the colonization rate of 100%. However, there were 16 outliers on the right upper corner of the figure 2.3. Population characteristics and patterns of association of these 16 outliers are shown in the table 25. The common factors among these 16 outliers included; the healthy population, there is no protective effect of X on A_1 (beta-coefficient =0.0000001) but there is small effect of X on B (beta-coefficient =0.69314718).

5. DISCUSSION

Based on data from our study, an effect estimate of X on Y_1 that that was identified by the CC approach and an effect estimate of X on Y_2 or Y_3 that was identified by the CCC approach were similar to those identified from the entire cohort population. Percent bias and coverage probability are within an acceptable range in nearly all clinical situations.

Our study revealed that the effect of prior FQ exposure (X) on the FQR infection identified by the CC approach is remarkably different from those identified by the CCC approach. The

difference was more pronounced if the study was conducted in healthy population, with a colonization rate of 10%, no protective effect of X on A_1 but there is small effect of X on B.

These findings support the results in previous literatures[83,84] which concluded that the CC approach almost always overestimates the effect of previous antibiotic exposure. Given that the difference between the CC and the CCC approaches would be significant only in situations that the protective effect of X on A_1 does not exist. Additionally, the number of subjects who have FQS and FQR colonization should be very low in the healthy population with a low colonization rate. Therefore, the difference between these two approaches is more pronounced.

Our study had some potential limitations. First, this study aimed to identify risk factors for antimicrobial resistance, the association between outcome and other factors were specifically designed by using the conceptual framework of emergence of antimicrobial resistance. Furthermore, most of pre-specified values were set by using data from antimicrobial resistance literature. Therefore, the results from our study might not be applicable to other kind of research.

Second, our study sample size was set at 200 subjects per group. This may be too small in some clinical situations. However, the average sample size of previous studies of antimicrobial resistance was 100-500. Therefore, this number seems to be similar to majority of previous studies's sample size. Third, our study methods were quiet complicated. The study results are probably too difficult to understand without basic knowledge in statistics.

In conclusion, our study confirmed that the CC approach and the CCC approach are not interchangeable. The CC approach could provide answers to assist clinicians in selecting antibiotic therapy before the full susceptibility profile of the pathogen is known. The CCC approach seems to be more useful for healthcare epidemiologists to implement an effective program to prevent spreading of resistant organisms. Researchers should carefully choose the appropriate study approach to best answer their research questions. However, our study was specifically designed for the study investigating risk factors for antimicrobial resistance, therefore the results may be different in other field of research. Future study comparing the CC vs. the CCC approach in other fields is still needed.

6. TABLES

Table 19. Characteristics of synthetic cohort population

Baseline characteristics	Healthy population	Sick population
Population size	100000	100000
Sample size	200	200
Colonization rate	10%, 50, 100%	10%, 50%, 100%
Prevalence of infection among the population	5%	10%
Prevalence of resistant infection among all infections	10%	20%
Prevalence of FQ exposure	10%	20%
Mean age +/-SD (year)	60+/-10	60+/-10
Prevalence of having at least one underlying diseases	10%	20%

Table 20. Variation in the colonization rate and the proportion of mechanism-2: mechanism-3

Variation	Colonization rate	Proportion of mechanism-2: mechanism-3
1	10%	10%:90%
2	10%	50%:50%
3	10%	100%:0%
4	100%	10%:90%
5	100%	50%:50%
6	100%	100%:0%

Table 21. Possible patterns of association and their corresponding beta-coefficients

Association between		Range of degree of association (beta-coefficient)			
Dependent variables	**Independent variables**				
C_1	C_2			0.69314718	
X	C_1			0.69314718	
X	C_2			0.69314718	
A_1	C_1			0.69314718	
A_1	C_2			0.69314718	
A_2	C_1			0.69314718	
A_2	C_2			0.69314718	
A_3	C_1			0.69314718	
A_3	C_2			0.69314718	
A_1	X	-0.69314718	0.00000001		
A_2	X		0.00000001	0.69314718	
A_3	X		0.00000001	0.69314718	1.0986123
B	X	-0.69314718	0.00000001	0.69314718	

Note: Exp (-0.69314718) = 0.5
Exp (0.00000001) = 1.0
Exp (0.69314718) = 2.0
Exp (1.0986123) = 3.0

Table 22. Comparison of the estimated effect and the true effect

Parameters to be evaluated	Comparison between the estimated value vs. the true value		
	Model-1: (FQR vs. FQS)	**Model-2 (FQR vs. No infection)**	**Model-3 (FQS vs. No infection)**
Mean percent bias (±SD)	6.47 ± 3.79	5.58 ± 2.27	4.75 ±2.23
Median percent bias (range)	5.51 (-0.75 - 22.73)	5.39 (-0.2 - 11.7)	4.47 (0.56 - 12.1)
Mean coverage probability (±SD)	0.96 ± 0.01	0.95 ± 0.01	0.95 ± 0.01
Median coverage probability (range)	0.96 (0.93 - 0.99)	0.95 (0.92 - 0.98)	0.95 (0.93 - 0.97)

Table 23. Population characteristics and patterns of association of clinical situations that provide those 5 outliers in the figure 3 (the OR of X on Y_1 (model-1) with a percent bias of 20% or more)

Clinical situations	**Population characteristics**	**Colonization rate (%)**	**Proportion A_2:A_3**	**Beta-coefficient between 2 variables***				**Percent bias**
				A_1-X	A_2-X	A_3-X	B-X	
409	Healthy population	10	0.5:0.5	0.01	0.01	0.69	0.69	22.73
337	Healthy population	10	0.1:0.9	0.01	0.01	0.69	0.69	21.98
403	Healthy population	10	0.5:0.5	0.01	0.69	0.01	0.69	21.11
304	Healthy population	100	0.5:0.5	0.01	0.01	0.01	0.69	21.06
340	Healthy population	10	0.1:0.9	0.01	0.01	0.01	0.69	20.10

Note: * approximated values

Table 24. Population characteristics and patterns of association of clinical situations that provide those 19 outliers in the figure 4.1 **(Healthy population)**

Clinical situations	Population characteristics	Colonization rate (%)	Proportion A_2:A_3	Beta-coefficient between 2 variables*				Percent bias
				A_1-X	A_2-X	A_3-X	B-X	
259	Healthy population	100	1	0.01	0.69	0.01	0.69	219.75
268	Healthy population	100	1	0.01	0.01	0.01	0.69	223.70
304	Healthy population	100	0.5	0.01	0.01	0.01	0.69	219.67
325	Healthy population	10	0.1	0.01	0.69	1.09	0.69	241.73
331	Healthy population	10	0.1	0.01	0.69	0.01	0.69	253.27
334	Healthy population	10	0.1	0.01	0.01	1.09	0.69	243.85
337	Healthy population	10	0.1	0.01	0.01	0.69	0.69	270.16
340	Healthy population	10	0.1	0.01	0.01	0.01	0.69	265.32
361	Healthy population	10	1	0.01	0.69	1.09	0.69	245.84
367	Healthy population	10	1	0.01	0.69	0.01	0.69	267.01
370	Healthy population	10	1	0.01	0.01	1.09	0.69	239.58
373	Healthy population	10	1	0.01	0.01	0.69	0.69	251.12
376	Healthy population	10	1	0.01	0.01	0.01	0.69	256.80
397	Healthy population	10	0.5	0.01	0.69	1.09	0.69	244.21
400	Healthy population	10	0.5	0.01	0.69	0.69	0.69	268.21
403	Healthy population	10	0.5	0.01	0.69	0.01	0.69	263.66
406	Healthy population	10	0.5	0.01	0.01	1.09	0.69	253.34
409	Healthy population	10	0.5	0.01	0.01	0.69	0.69	274.74
412	Healthy population	10	0.5	0.01	0.01	0.01	0.69	263.81

Note: * approximated value

Table 25. Populations characteristics and patterns of association of clinical situations that provide those 16 outliers in the figure **5.1**

Clinical situations	Population characteristics	Colonization rate (%)	Proportion A_2:A_3	Beta-coefficient between 2 variables*				Percent bias
				A_1-X	A_2-X	A_3-X	B-X	
325	Healthy population	10	0.1	0.01	0.69	1.09	0.69	241.73
331	Healthy population	10	0.1	0.01	0.69	0.01	0.69	253.27
334	Healthy population	10	0.1	0.01	0.01	1.09	0.69	243.84
337	Healthy population	10	0.1	0.01	0.01	0.69	0.69	270.16
340	Healthy population	10	0.1	0.01	0.01	0.01	0.69	265.31
361	Healthy population	10	1	0.01	0.69	1.09	0.69	245.84
367	Healthy population	10	1	0.01	0.69	0.01	0.69	267.01
370	Healthy population	10	1	0.01	0.01	1.09	0.69	239.57
373	Healthy population	10	1	0.01	0.01	0.69	0.69	251.12
376	Healthy population	10	1	0.01	0.01	0.01	0.69	256.79
397	Healthy population	10	0.5	0.01	0.69	1.09	0.69	244.21
400	Healthy population	10	0.5	0.01	0.69	0.69	0.69	268.20
403	Healthy population	10	0.5	0.01	0.69	0.01	0.69	263.66
406	Healthy population	10	0.5	0.01	0.01	1.09	0.69	253.34
409	Healthy population	10	0.5	0.01	0.01	0.69	0.69	274.74
412	Healthy population	10	0.5	0.01	0.01	0.01	0.69	263.81

Note: * approximated value

7. FIGURES

Figure 1. Mechanisms of developing infection

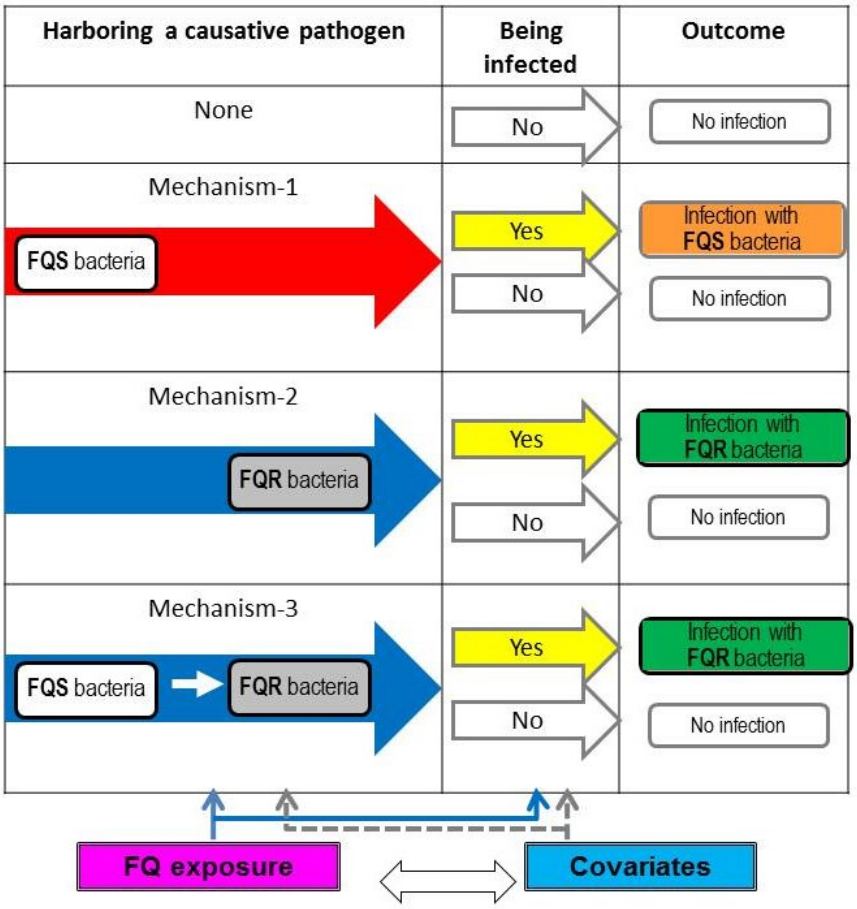


Figure 2. Structure of the synthetic cohort population

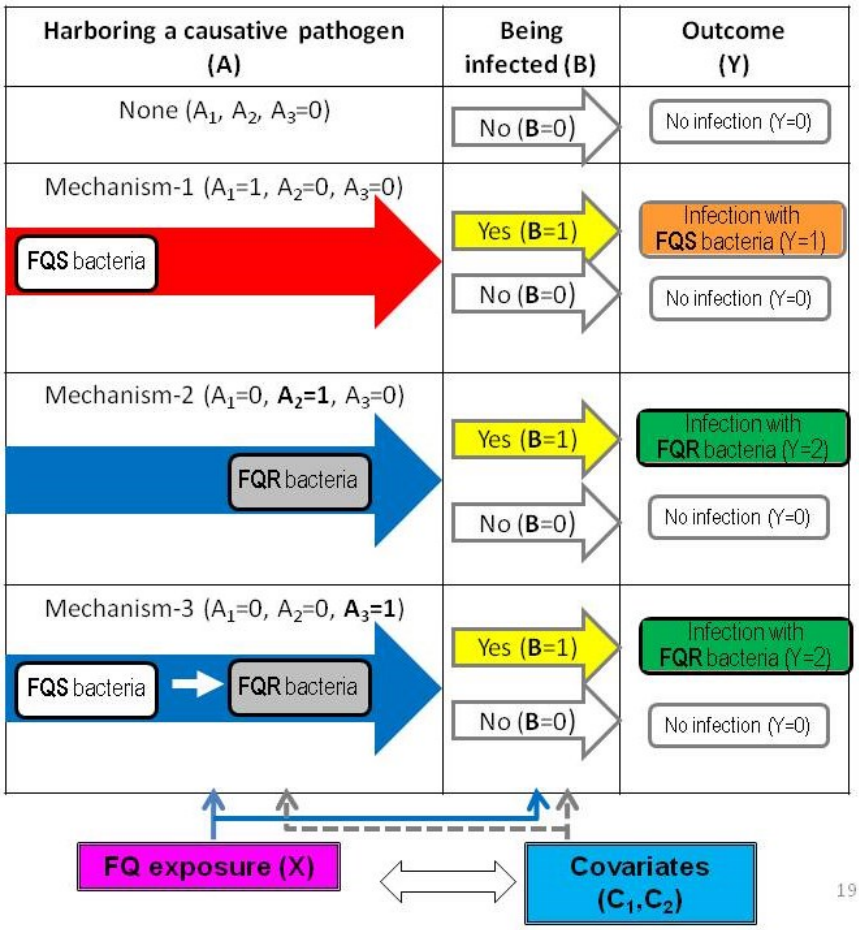


Figure 3. Percent deviation between the estimated OR_{Y1} and the estimated OR_{Y2} across all clinical situations

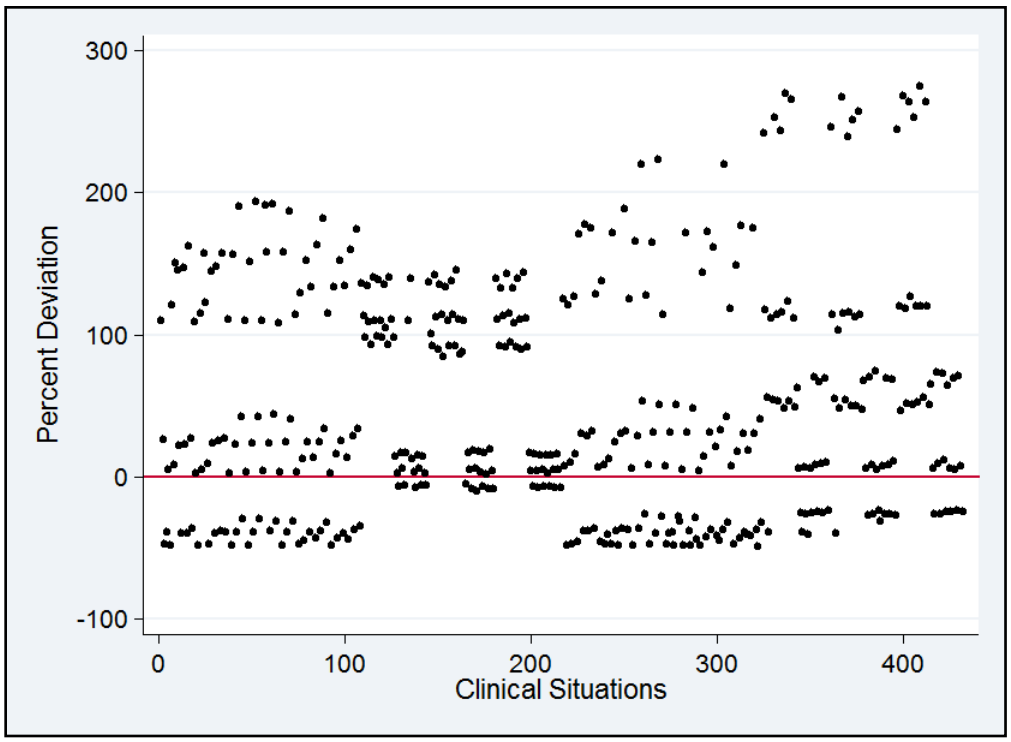


Figure 4 Percent deviation by the population type (Healthy vs. Sick population)

Figure 4.1 Healthy population, by patterns of association

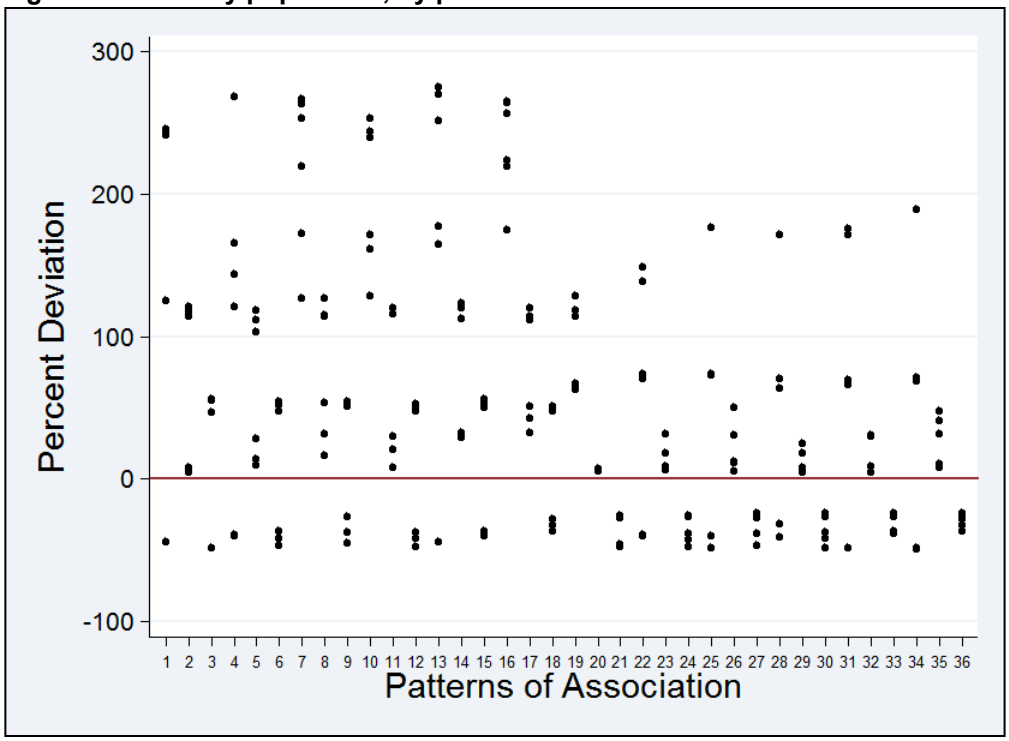


Figure 4.2 Sick population, by patterns of association

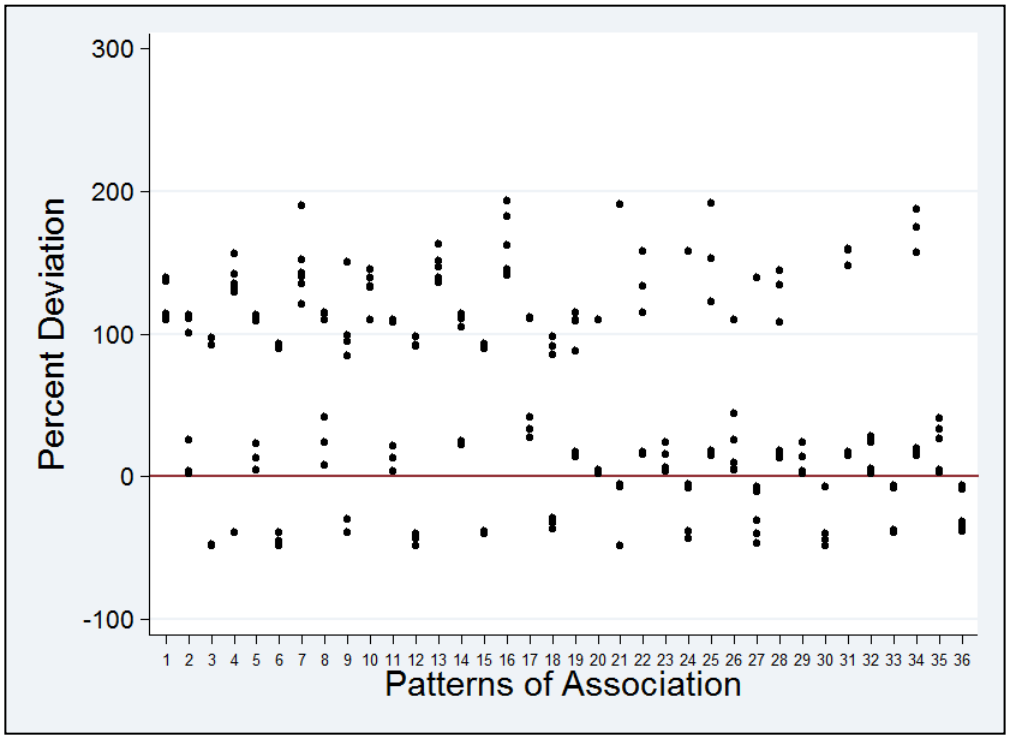


Figure 5 Percent deviation by the colonization rate (10% vs. 100%)

Figure 5.1 Colonization rate =10%, by patterns of association

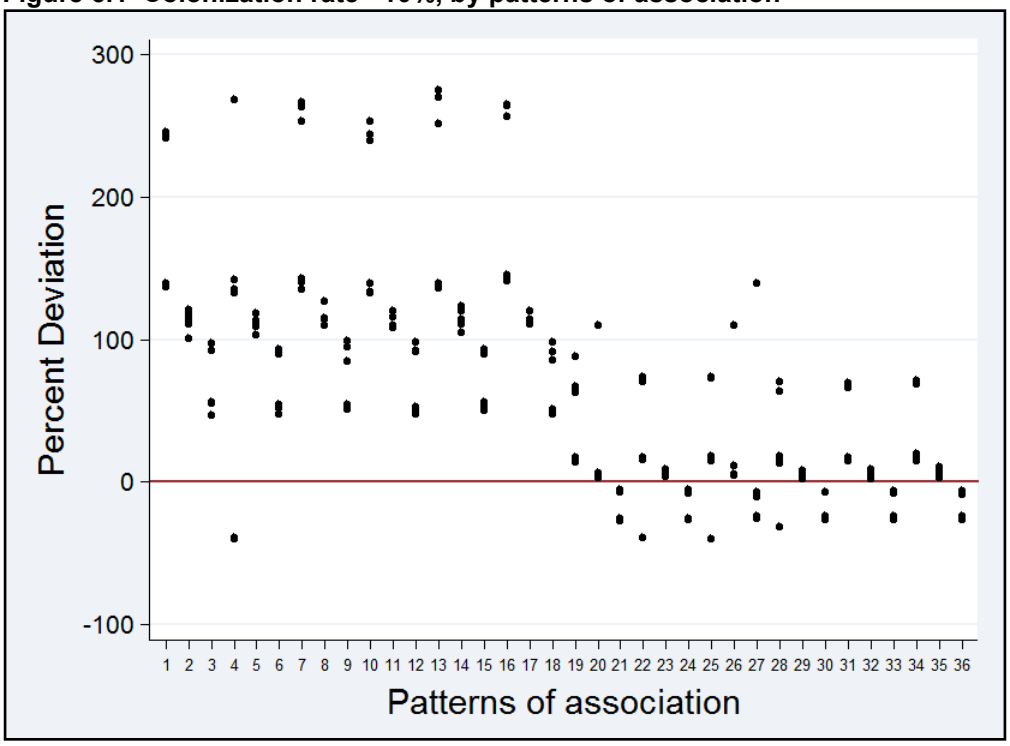
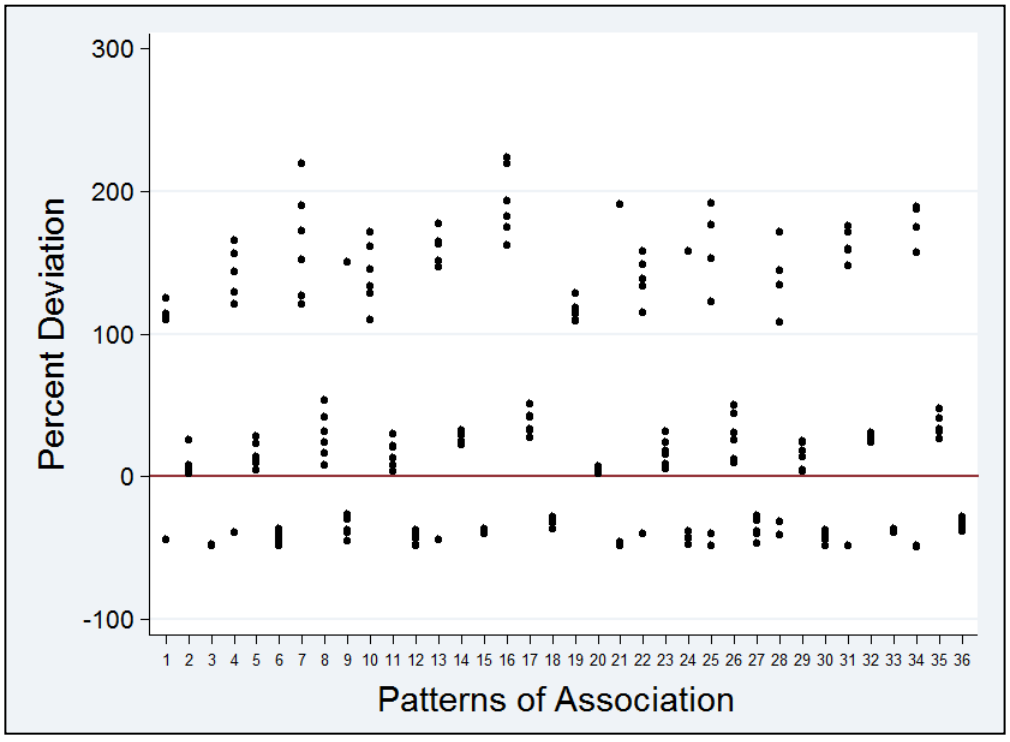


Figure 5.2 Colonization rate =100%, by patterns of association



DISSERTATION CONCLUSION

This dissertation project consisted of three related studies exploring the problem of emergence of fluoroquinolone resistance. Two content-based studies focused on the high MIC fluoroquinolone-susceptible *E. coli* (FQSEC) which may serve as an important reservoir for FQ resistance. A method-based study evaluated two case-control approaches in investigating risk factors for antimicrobial resistance.

The first study was a case-control study conducted to identify risk factors for high MIC-FQ susceptibility among female subjects with urinary tract infections (UTIs) caused by FQSEC. Independent risk factors identified in this study included Asian race, having renal diseases and previous exposure to nitrofurantoin. These findings could not be explicitly explained. Future studies need to be done to explore these interesting findings.

The second study was a cohort study of female subjects with ambulatory FQSEC-UTIs who were treated with FQ therapy. Adjusted analysis revealed that treatment failure in the high MIC group was 8 times higher than those in the low MIC group. Therefore, the current MIC breakpoint for FQ susceptibility in *E. coli* uropathogen may need to be revised. Our study results would be useful for those reevaluating breakpoints.

In the method-based study, we quantitatively compared the conventional case-control (CC) approach and the novel case-case-control (CCC) approach in investigating risk factors for infection caused by FQ-resistant pathogen in the simulated setting. Our study confirmed that the CC approach almost always overestimates the effect of previous antibiotic exposure. The difference is more pronounced if the study is to be conducted among healthy population with a lower rate of colonization and protective effect of exposure on mechanism of harboring FQ-susceptible pathogen does not exist. Therefore, researchers should carefully choose the appropriate study approach to best answer their research questions.

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