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Identifying new risk factors for antimicrobial resistance in urinary tract infections through microbial, genotypic, and geospatial analyses

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Identifying new risk factors for antimicrobial resistance in urinary tract infections through microbial, genotypic, and geospatial analyses

By

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ABSTRACT

Identifying new risk factors for AMR UTI through microbial, genotypic, and geospatial analyses

by

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Antimicrobial resistance and the subsequent loss of effective antimicrobial treatments is one of the greatest threats facing public health in the 21st century. The increasing prevalence of antimicrobial resistant (AMR) bacteria threatens the effective treatment of common and previously easily treated bacterial infections, such as urinary tract infections (UTIs), and if unaddressed could effectively turn back the clock to a pre-antibiotic era. The complex dynamics of horizontal gene transfer, selective pressure from antimicrobial agent usage and the dissemination of resistant bacterial strains, present a multifaceted problem that will challenge researchers and policy makers, requiring interdisciplinary problem-solving and political will. To slow the spread of resistant strains and limit the impact of antimicrobial resistance selection, global and local public health agencies will require detailed surveillance and improved diagnostic efforts to guide clinical practice.

This work attempted to address some of these concerns by examining potential risk factors for AMR UTI through genotypic and geospatial analysis. Our goal was to improve understanding of AMR UTI by leveraging whole genome, patient level and geospatial data. We examined co-resistance of bacteria causing UTI and identified genotypic candidates for resistance markers that may aid in both diagnostic development, as well as surveillance. We also investigated geospatial clustering of UTI by examining common *Escherichia coli* strains associated with AMR UTI within the community and examined patient characteristics associated with common strains. These approaches to characterize the epidemiology of AMR UTIs have challenged the traditional view that the community prevalence of these infections is largely due to antibiotic selective pressure on *E. coli* pathogens. Our observations demonstrate that a large proportion of community-onset AMR UTIs are caused by strains of *E. coli* that are already resistant at the time of infection. This body of work provides the foundation for future investigations of AMR UTI risk factors that will help provide a basis for public health surveillance and intervention.

I would like to dedicate this work to Dr. Lee W. Riley, my brilliant mentor. Rest assured; I'll continue to work on the problems of this world. I hope you know you made a real difference here, and I'm hopeful knowing you've already started working to solve the problems of whatever universe comes next.

I would also like to dedicate this work to Deborah Johnston, a woman who never failed to make me feel loved and supported. Thank you for bringing such a bright light into this world, he is my guiding star. We will miss you dearly.

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INTRODUCTION

Since the discovery and proliferation of drugs like penicillin in the 1940's, modern medical practice has been revolutionized by access to antimicrobial agents. They have allowed for the fast and effective treatment of common and previously life-threatening infections and have made many surgical and oncological treatments possible.[1] Antimicrobial agents have also been heavily utilized in modern agriculture, playing a central role in the prevention and treatment of infections in increasingly dense farming operations, and increasing animal growth efficiency.[1] The use of antibiotics is truly pervasive in the US. In fact, the U.S. Centers for Disease Control and Prevention (CDC), recently reported that 80% of Americans will be prescribed an antibiotic each year. Global measures have estimated an antibiotic consumption rate of 14.3 defined daily doses (DDD) per 1000 people per year, demonstrating an increase of 46% from year 2000.[10] Therefore, it is of the utmost importance that as one of our most important medical tools, antibiotics remain effective.[2]

Antimicrobial resistance (AMR) occurs through a natural process by which bacteria acquire mechanisms to counteract antimicrobial agents. These mechanisms increase in prevalence amongst bacterial populations primarily through horizontal gene transfer, selective pressure from antimicrobial agent usage leading to advantageous gene mutations, and through the dissemination of previously resistant bacterial strains.[1] The rapid spread of AMR in bacteria represents a substantial threat to public health and to numerous medical and agricultural advances. In 2019, it was estimated that 1.27 million annual global deaths were attributable to bacterial AMR. Furthermore, it is estimated that by 2050, AMR deaths will increase to 1o million deaths annually worldwide, making it a strong candidate for the future leading cause of death worldwide. [3]

Urinary tract infections (UTI) are the most common bacterial infection in women and are a leading cause of the prescription of oral antibiotics.[4] UTI are the most frequent infection in long term care facilities and account for a third of all skilled nursing facility associated infections.[6] These infections contribute significantly to disease burden in hospital settings, and are second only to respiratory infections as leading causes of secondary infections. They also represent a considerable burden to patient quality of life, with 27% of patients experiencing UTI recurrence after their first episode of UTI within the first 6 months. [7] In fact, it is estimated that the health care costs associated with acute cystitis in the United States alone is 3.5 billon dollars each year. Left untreated, complicated cases of UTI can lead to several severe outcomes such as disseminated

blood stream infections. Each year it is estimated that approximately 1.7 million adults in the US develop sepsis, a life-threatening complication of infection, with a higher proportion of deaths experienced by adults over the age of 65.[5] In fact, 30% of sepsis cases are estimated to have a UTI origin.[6] UTI in elderly populations can have serious impacts on overall health and life expectancy, as they lead to incontinence, delirium, decreased mobility, falls, and altered mental status.[5]

UTI have been known to be caused by many opportunistic pathogens, but the dominant organism causing hospital acquired (HA) and community acquired (CA) infections is uropathogenic *Escherichia coli* (UPEC). In fact, UTI caused by UPEC account for over 70% of all UTI infections in the United States. Antimicrobial resistance (AMR) is a growing public health concern in the clinical management of UTI. The high prevalence of AMR UTI in health care settings is a widely recognized public health threat, but increased prevalence of AMR UTI in community settings have recently become a cause for alarm amongst public health experts.[4]

There are many mechanisms of resistance to various classes of antimicrobials. In this work, we will focus primarily on resistance to β-lactam antibiotics. β-lactams are a group of antimicrobial agents which act upon the cell wall, and include common drug classes like cephalosporins, monobactams, carbapenems and penicillins. β-lactam antibiotic use is highly pervasive. It has been estimated that in the United States, they account for approximately 65% off all antibiotics prescribed. Resistance to β-lactam antibiotics has become increasingly common in UPEC and the most common mechanism of resistance to β-lactam antibiotics is through the production of β-lactam hydrolyzing enzymes called β-lactamases.[7] Extended spectrum β-lactamase (ESBL) producing *E. coli* bacteria pose a real public health threat both to CA and HA infections.

In order to address the threat of AMR, both the CDC and the World Health Organization (WHO) have released reports that suggest the need for increased efforts to combat AMR, focusing on ESBL and carbapenem resistance in Gram-negative bacteria, like *E. coli*. The strategies they suggest are fourfold: 1) infection control and prevention, 2) improved surveillance of AMR, 3) stewardship promotion and improved prescribing practices and 4) development of AMR diagnostics and new antimicrobial agents.[8,9] The application of these strategies is of immense consequence for the clinical treatment of UTI, as it is the most common ailment for which antibiotics are prescribed. Therefore, to address the global spread of AMR, it is imperative that we understand AMR UTI.

This body of work first attempts to expand our understanding of risk factors for AMR UTI through systematic literature review, focusing on the role that environmental sources of ESBL UPEC may play in CA UTI. Next, this work attempts to broaden understanding of co-resistance within organisms causing UTI which are resistant to third generation cephalosporins through an analysis of a multicenter patient population. This project uses phenotypic and genotypic data from patient bacterial isolates to investigate risk of co-resistance, while investigating potential genetic markers that could be used for improved AMR surveillance and diagnostic development with the potential to improve prescribing practices. The final chapter of this work focuses on the dissemination of resistant strains of UPEC causing UTI within San Francisco County. By genotyping patient isolates and using patient addresses, we identified strain specific clustering of UTI. Through these investigations we have elucidated aspects of AMR UTI transmission and helped to define novel potential targets for AMR diagnostics and surveillance. It is our hope that through this work we will contribute to the global effort to combat the rise of AMR.

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CHAPTER ONE. Risk factors associated with community-acquired urinary tract infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*: a systematic review

INTRODUCTION

Community-acquired urinary tract infections (CA-UTI) are the most prevalent bacterial infection in women and are, overall, the most common reason for the prescription of oral antibiotics[1]. It is estimated that approximately 150 million people worldwide develop urinary tract infections every year. Of the many pathogens known to cause these infections, the most common is uropathogenic *Escherichia coli* (UPEC) [2].

Antimicrobial resistance is a growing public health concern in the clinical management of CA-UTI caused by UPEC. Multidrug-resistant *E. coli* are of increasing concern due to their associated high rates of treatment failure and large economic burden [1]. CA-UTI is treated empirically, and in the United States, the Infectious Disease Society of America (IDSA) recommends the use of nitrofurantoin, trimethoprim/sulfamethoxazole (TMP-SXZ), or fosfomycin for the treatment of acute uncomplicated cystitis/UTI [3]. While most UPEC strains in the US remain susceptible to nitrofurantoin, this drug is recommended only for acute uncomplicated cystitis and cannot be used for more severe infections such as pyelonephritis. The increasing frequency of resistance of UPEC to TMP-SXZ and fluoroquinolones, as well as earlier-generation beta-lactam drugs (ampicillin, cephalexin) have led to greater use of broader-spectrum beta-lactam antimicrobial agents in many regions of the world [4].

Extended-spectrum β-lactamase (ESBL) producing organisms are characterized by their ability to produce enzymes that hydrolyze third-generation cephalosporins and aztreonam [5]. ESBL-producing Enterobacteriaceae organisms, including *E. coli*, are considered "serious threat" hazard level pathogens by the US Centers for Disease Control and Prevention [6]. ESBL-producing *E. coli* are frequently resistant to multiple classes of antimicrobial agents in addition to beta-lactams.

In the NCBI PubMed database, the term "extended-spectrum beta-lactamase-producing *E. coli*" first appeared in a publication from 1990 [6]. UTIs caused by ESBL-producing *E. coli* are becoming increasingly common amongst community-acquired infections [7]. These cases began to be reported in community onset infections in 1998, first in Ireland, Israel, Spain and France [8]. However, factors that contribute to this increasing prevalence of ESBL-producing UPECs in communities are not clearly understood. A better understanding of risk factors associated with such infections is needed to improve clinical management of ESBL UPEC infections.

The widespread clinical use of antimicrobial agents has resulted in the selection of antimicrobial drug-resistant (AMR) bacterial strains, and yet, it remains unclear if this factor alone contributes to the widespread dissemination of AMR UPECs in community settings. Population-based studies of CA-UTI have shown that a large proportion (>50%) of AMR infections are caused by just five to six lineages of *E. coli*, referred to as pandemic extraintestinal pathogenic *E. coli* (ExPEC) lineages, defined by multilocus sequence typing (MLST) [9,10]. Therefore, the community prevalence of AMR CA-UTI may be largely influenced by ExPEC genotypes circulating in a community, instead of initial selection of resistant strains by antimicrobial agents. Thus, UPEC resistance selection alone appears to be insufficient to determine community prevalence of AMR CA-UTI.

Most studies that assess risk factors for AMR infections focus on factors related to the selection of AMR strains and inevitably identify host-related factors, such as previous use of antibiotics, underlying medical conditions (e.g., diabetes, urinary tract anatomical defects) or medical procedures (e.g., catheterization). These studies do not take into consideration risk factors related to transmission and sources of AMR UPEC organisms. The observation that a large proportion of AMR CA-UTIs in a community may be caused by only a few ExPEC lineages suggests that there are point sources of these strains to which people are exposed. There is growing evidence that a substantial proportion of CA-UTI may be caused by UPEC strains that contaminate food products [11–16]. Food animals may serve as this point source. In major food exporting countries, antimicrobial agents are used in large quantities in animal husbandry to prevent infectious disease and promote growth [17–19]. Hence, it is conceivable that AMR UPECs are initially selected in such animal reservoirs, contaminate human food and the environment, and ultimately enter the human intestine and then enter the bladder to cause UTI. One question posed in this review, therefore, is whether ESBL-producing UPECs originate from sources outside of the human host.

This review is divided into two parts. The first part considers the current body of knowledge regarding risk factors for CA-UTI caused by ESBL-producing UPEC. We then undertake a more detailed examination of potential risk factors for transmission of ESBL-producing UPEC by reviewing the current body of knowledge that addresses the role of food and environmental sources for ESBL-producing ExPECs that cause CA-UTI. We identify new risk factors for transmission of ESBL-producing UPECs with the hope to inform research agendas, public policy, and public health intervention strategies.

MATERIALS AND METHODS

Data sources and search strategy

Two independent authors conducted two literature searches using the databases PubMed, Embase, and Web of Science. In both queries, we limited the search to articles published between 2014 to 2019, the five most recent years. To include highly influential works published before this time period, we included articles published before 2014 that were cited greater than 50 times. Only articles published in English were included.

The first search focused on risk factors for CA-UTI caused by ESBL-producing UPEC and was conducted on January 8th, 2019. It included the search terms: ("Community-Acquired Infections"[Mesh] OR "Community-Acquired") AND ("Urinary Tract Infection*" OR "Urinary Tract Infections"[Mesh]) AND ("Drug Resistance, Microbial"[Mesh] OR "antimicrobial resistance" OR "antibiotic resistant ") ('urinary tract infection' OR 'urinary tract infection'/exp) AND ('community acquired' OR 'community acquired infection'/exp) AND ('antibiotic resistance'/exp OR 'antibiotic resistance' OR 'antimicrobial resistance'). Our second search, which focused on ESBL-producing ExPECs found in food and environmental sources, was conducted on April 8th, 2019 and included the search terms; ('extraintestinal pathogenic escherichia coli'/exp OR 'extraintestinal pathogenic escherichia coli') AND ('antibiotic resistance'/exp OR 'antibiotic resistance' OR 'antimicrobial resistance'/exp OR 'antimicrobial resistance' OR 'antibiotic resistant') AND ('food'/exp OR 'food' OR 'animal'/exp OR 'animal' OR 'environment'/exp OR 'environment').

Study selection and data extraction

Risk factors for ESBL-producing CA-UTI

All study abstracts were reviewed by each of the two independent authors and were considered to be eligible for inclusion for full text review if they reported risk factors associated with CA-UTI caused by ESBL-producing *E. coli*. Studies deemed relevant by both authors were reviewed in full. All disagreements were resolved by consensus prior to proceeding to data extraction. Study populations comprised of both adult and pediatric patients were included (Figure 1).

Data extraction was conducted by two independent authors and exported to a single Excel spreadsheet for evaluation. Relevant recorded information included author names, year of publication, sample size, location of study, study design, risk factors investigated, outcome measurement methods, statistical analysis methods and limitations. We evaluated evidence in support of possible risk factors by examining total number of significant study findings, sample size and author-listed limitations. Risk factors were deemed to be "commonly assessed" if they were included in more than three studies (Table 2).

Sources of ESBL-producing ExPEC

Study abstracts were reviewed by two independent authors and were considered to be eligible for inclusion for full text review if they reported analyzing ESBL-producing ExPEC isolates from food, animal, or environmental sources and included multilocus sequence typing (MLST) data based on the Achtman scheme [20]. Studies deemed relevant by both authors were reviewed in full, and all disagreements were resolved by consensus prior to proceeding to data extraction (Figure 1).

Data extraction was conducted by two independent authors and exported to a single Excel spreadsheet for evaluation. Relevant recorded information included author names, year of publication, sample size, location of study, study design and ExPEC sequence types.

RESULTS

Risk factors for ESBL-producing CA-UTI

The initial multi-database search query returned 414 studies matching search criteria; 88 were removed as duplicates and 326 abstracts were reviewed. Two hundred and fifty-eight studies were deemed to be irrelevant by two independent researchers and 68 studies were reviewed in full. Fifty-three studies were excluded: 51 for wrong outcomes measured or non-CA-UTI comparator, 1 for being a review, and 1 for being a non-English publication. This review focuses on 15 studies that examined 60,924 patient urine samples that included 2,930 ESBL-producing *E. coli* infections.[21,22,31–35,23–30] Of these 15 studies, eight used case control, three used prospective cohort, and four used retrospective cohort study designs. The number of cases caused by ESBL-producing UPEC examined in each study ranged from 21 to 1694. Study settings included hospital inpatient, community clinic, and hospital outpatient services. The majority of studies took place in Europe (8), while other study locations included South Korea (2), Thailand (1), China (1), Peru (1), Mexico (1), Turkey (1), and Jordan (1). Table 1 lists all studies identified with relevant characteristics. Studies compared cases (CA-UTI caused by ESBL-producing *E. coli*) with controls defined as CA-UTI caused by non-ESBL producing *E. coli* (14) or CA-UTI caused by non-ESBL producing *E. coli* resistant to at least one antibiotic (1). There were 103 unique risk factors

assessed by all identified studies. Of the 103 risk factors, 8 were deemed "commonly assessed" by this review. The most frequent risk factors assessed were: previous hospitalization (11), antibiotic use within the past three months (9), male sex (9), pre-existing condition of type II diabetes (5) previous UTI (6), recurrent UTI (5), previous catheterization (5), and urinary tract abnormality (4). Evidence in support of potential risk factors was commonly reported using odds ratios (OR), with significance reported as 95% confidence intervals (CI) and associated p-values. Common statistical methods included univariate logistic regression, multivariate logistic regression, chi-squared test and Fisher's exact t-test.

Statistically significant associations between commonly assessed potential risk factors and CA-UTI caused by ESBL-producing *E. coli* were found to vary between studies. For example, seven (78%) of nine studies found a statistically significant association between antibiotic use in the past three months. Male sex was found to be a significant risk factor for ESBL producing *E. coli* infection in five (56%) of nine studies. Previous hospitalization was the most common risk factor examined across studies and was found to be a significant potential risk factor in 8 (73%) of 11 studies. Previous UTI caused by any organism was also found to be significantly associated with CA-UTI caused by ESBL-producing *E. coli*, reported in six of six studies. Recurrent UTI, defined as 3 episodes of UTI in the previous 12 months, was found to be a significant potential risk factor in 5 of 5 studies. The potential risk factors with the highest percentage of patients who were infected with ESBL-producing UPEC were previous UTI (79%) and previous catheterization (60%) (Table 2).

Sources of ESBL-producing ExPEC

The initial multi-database search query returned 514 studies matching our criteria; 99 were removed as duplicates and 415 abstracts were reviewed. Three hundred seventy-one studies were deemed irrelevant by two independent researchers and 44 studies were reviewed in full by each author. Twenty-five studies were excluded: 23 for wrong outcomes reported, 1 for being a review and 1 because it was not published in full. This review focuses on 19 studies that examined 2042 ESBL-producing ExPEC specimens isolated from environmental, food or animal sources [36,37,46–54,38–45]. Sources of ExPEC sampled included vegetables (1), houseflies (1), dogs (7) cats (7), horses (2), cattle (3), chicken (3), and water samples (2). Two thousand forty-two ESBL-producing ExPEC isolates were recovered. Sixteen (84%) of 19 studies reported ESBL producers of ExPEC pandemic lineages (ST10, ST69, ST73, ST95, ST127, ST131)[55].

In the course of this review, all six pandemic UPEC lineages with evidence of ESBL production were discovered. The most commonly recovered pandemic sequence type was ST131, which appeared in 12 (63%) of 19 studies, followed by ST10 which appeared in 7 (37%) of 19 studies (Figure 1).

DISCUSSION

We found conflicting reports of factors associated with CA-UTI caused by ESBL-producing *E. coli*. The reviewed studies were not concordant for significant association in six of the eight commonly assessed risk factors. This may have resulted from differences in types of variables sought, definitions used, sample size, and other factors associated with study design. Conversely, this review found agreement in all studies for previous UTI episodes and recurrent UTI as potential risk factors for UTI caused by ESBL-producing *E. coli* (Table 2). These risk factors are not new

as they can serve as risks for any AMR infections. The above studies identified only risk factors associated with host-related characteristics. Previous UTI episodes and recurrent UTI may represent the same type of disease occurrence and likely select for AMR UPECs because of repeated exposures to antimicrobial agents. However, factors associated with selection of AMR UPECs are outside of the scope of this review.

In this review, we wished to identify factors associated with the increasing global prevalence of CA-UTI caused by ESBL-producing UPECs. Selection of AMR UPEC strains does not necessarily lead to increased community prevalence of AMR CA-UTI. Additionally, only 1 of the 15 studies used a comparison group that included only AMR CA-UTI caused by non-ESBL producing *E. coli* [31]. The remaining studies used controls which were defined as infection caused by non-ESBL producing *E. coli* and did not explicitly exclude susceptible cases. This may impact the ability of such studies to distinguish risk factors for ESBL- producing infection from the more general risk factors for AMR.

None of the 15 studies selected in the first review examined risk factors associated with transmission of ESBL-producing UPEC, which would affect community AMR prevalence. An increasing number of studies have suggested that non-human reservoirs, such as food products, could serve as a potential source of human ExPEC exposure [11,13,16]. Historically, diseases such as CA-UTI are seldom described as occurring as outbreaks. However, recent molecular epidemiological investigations have revealed that many CA-UTI cases, which appear sporadic, are caused by distinct sets of *E. coli* genotypes, suggesting point or common source exposures [56,57]. Thus, UPEC may be acquired from contaminated food products or other external sources (e.g., water, environment) [10,13,58,59]. In fact, the ST69 UPEC lineage—as genotyped by MLST—was first suggested to disseminate by contaminated food in the US in 1999 [15]. More recently, one study found UPEC sequence type 131 recovered from poultry meat to be closely related to CA-UTI clinical isolates of ST131 by phylogenetic analysis and ColV plasmid interrogation [12]. By pulsed field gel electrophoresis (PFGE), raw poultry has been implicated as a possible source of human ExPEC strains in Canada [60], although these *E. coli* strains did not express ESBL. A study in the Netherlands discovered a high frequency of ESBL genes in *E coli* strains isolated from raw chicken samples, many of which were shown to be identical to those found in human rectal swabs and blood cultures [61]. Despite these data, the effect and magnitude of food or food animals as a source of ESBL-producing UPEC is yet to be well established.

Furthermore, among the publications included in this review, we found extensive evidence of the presence of ESBL producing pandemic UPEC lineages in food animals, companion animals and other environmental sources (Figure 1). These lineages are implicated in the vast majority of human cases of CA-UTI, which may suggest that there are common sources of these strains to which people are exposed. The relationship between food or food animals and AMR infections has been well established for enteric bacterial pathogens such as Salmonella, Campylobacter, and Shigatoxin-producing *E. coli* (STEC) [18,58,62–69]. Antibiotic use in animal husbandry is recognized as a key contributor to AMR selection in these enteric pathogens causing human gastrointestinal infections [17,70]. The prevalence of AMR enteric infections in communities, however, is largely influenced by outbreaks and dissemination of these enteric pathogens by contaminated food products. It is therefore not inconceivable that food animals and food products, which have been shown to be contaminated with ESBL producing *E. coli*, could cause CA-UTI and affect community prevalence of AMR CA-UTI [70–72]. Thus, exposures to certain types of food products or environmental sources may serve as important risk factors for CA-UTI caused by

ESBL-producing UPEC. The intensification of food animal production, expanding use of antimicrobial agents in animal husbandry, and globalization of food trade may be contributing to the increasing global prevalence of CA-UTI caused by ESBL-producing *E. coli*.

Study limitations: The vast majority of risk factors investigated in the first review represent individual level risk factors that utilize simple demographic and health record information. No studies captured by this review investigated potential community level exposure risk factors, which may play a role in a patient's risk for CA-UTI caused by ESBL-producing *E. coli* and affect community prevalence of such infections. Of the eight commonly assessed risk factors addressed here, finding association with factors such as age, sex and previous hospitalization provides limited opportunity for public health intervention. This demonstrates a clear need for studies that prioritize prevention of transmission as a motivation for risk factor investigation, as well as a need for new study designs that include strain genotype data that leverage community level data, such as places in the community for food product purchase and exposures.

Previous work on multidrug-resistant infections describe an existing need for standardization of risk factor definitions [73]. This review found that the coding of risk factor analysis varied dramatically between studies. For example, many of the evaluated risk factors represented similar exposures. However, inconsistency in the time since exposure made comparison impossible between otherwise categorically similar exposures. A meta-analysis of the full breadth of findings was therefore difficult, and subsequent recommendations for clinical practice harder to suggest.

Currently, there are few studies that simultaneously and prospectively compared human isolates of ESBL-UPEC with *E. coli* strains isolated from food and environmental sources from the same geographic sites. Sampling of food or environmental products in most studies are frequently under-powered to sufficiently demonstrate links. Larger systematically well-designed studies are required to determine if contaminated food or environmental products act as a vehicle of ESBL-producing UPEC that cause human CA-UTI.

As is the case with many literature reviews, our results are limited by potential publication bias, as part one of this review only examined journal articles that reported positive associations. Our conclusions are also limited by the exclusion criteria that restricted our scope to articles written in English. UPEC strain types may cluster geographically and temporally, suggesting that risk factors for UTI may vary geographically and by time [10,74]. Although our review includes studies from several regions of the globe, geographic differences in UPEC genotype distribution may impact the generalizability of the results of this review.

CONCLUSION

The risk factors we found for CA-UTI caused by ESBL-producing UPEC reported in the reviewed articles include broad categories that may not be specifically related to UPEC organisms that produce ESBL. Factors such as previous UTI episodes and recurrent UTI may represent risk factors for any drug-resistant UTI and not necessarily UTI caused by ESBL-producing UPEC. Such observations may result from our current lack of precise understanding of mode of transmission of CA-UTI. Furthermore, this review found compelling evidence of the presence of ESBL producing pandemic UPEC lineages that have been implicated in some human cases of CA-UTI in food animals, companion animals and other environmental sources. These results may suggest that there are in fact point sources of human exposure to these pathogens. Further studies

investigating these source exposures may generate new information that can be used to devise focused and effective public health interventions.

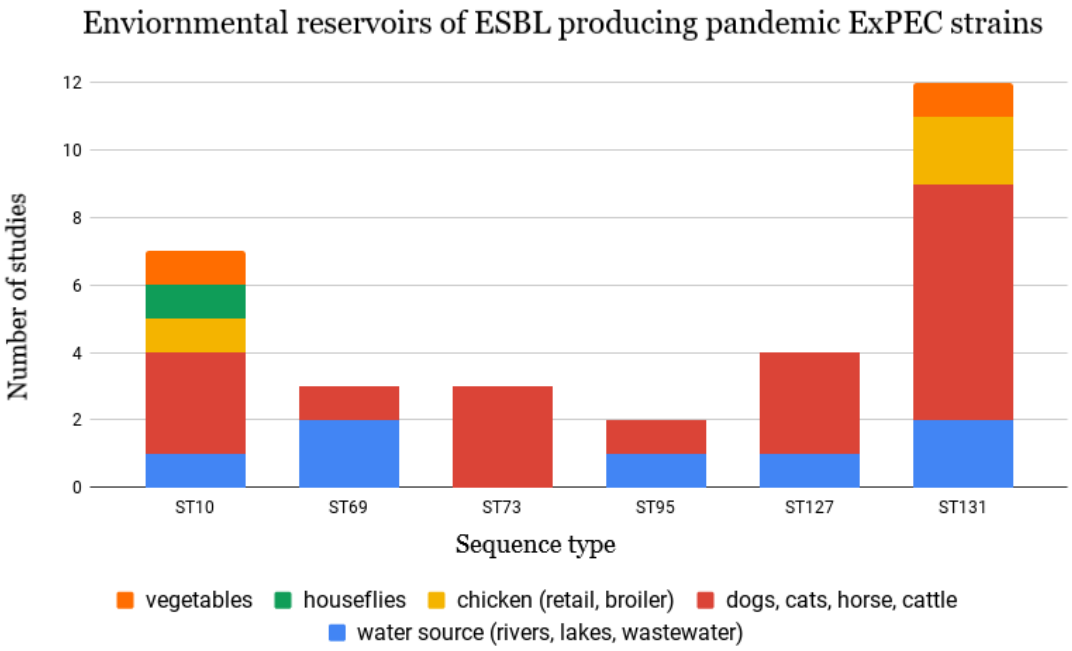
Table 1. Characteristics of studies included in review

Lead author, year of publication	Country	Setting or sample origin	Species	Study type (CC, R, P)	Number of samples assessed (n)	Total number ESBL positive (n)	% ESBL (%)
Risk factors for ESBL-producing CA-UTI							
Søgaard 2017	Denmark	Laboratory Information System/database	Human	CC	3390	339	9%
Sittichanbuncha 2016	Thailand	University Hospital	Human	R	399	159	40%
Pérez Heras 2017	Spain	Tertiary Care Hospital	Human	R	229	21	9%
Martin 2016	France	Private Practice laboratories	Human	R	51,643	1694	3%
Lee 2018	Korea	University Hospital	Human	CC	150	50	33%
Kim 2017	Korea	University Hospital	Human	R	186	31	17%
Jacmel 2017	France	Pediatric Emergency Department, Hospital	Human	P	403	22	5%
Hertz 2016	Denmark	University Hospital	Human	CC	442	98	22%
Hernández Marco 2017	Spain	Pediatric Hospital	Human	CC	537	19	4%
Fan 2014	Taiwan	Children's Hospital	Human	CC	312	104	33%
Chervet 2018	France	Parisian suburb laboratory platform	Human	P	849	36	4%
Castillo-Tokumori 2017	Peru	Main Hospital	Human	CC	1158	67	6%
Azap 2010	Turkey	Four Tertiary-care Hospitals	Human	P	269	17	6%
Almomani 2018	Jordan	University Hospital	Human	CC	591	251	42%
Alcántar-Curiel 2015	Mexico	Mexico's Naval Referral Hospital	Human	CC	70	22	31%
Sources of ESBL-producing ExPEC							
DeRauw 2019	Belgium	STEC infection	Calves	CC	9	1	11%
Ewers 2014	Europe	Naturally occurring infections	Mammals	P	1152	1152	100%
Ghodousi 2016	Italy	Retail chicken meat	Chicken	CC	237	237	100%
Ghodousi 2015	Italy	Retail chicken meat	Chicken	P	163	132	81%
Gomi 2015	Japan	Waste Water + Hospital Water	N/A	P	32	32	100%
Guo 2015	Australia	Feces and clinical isolates	Dog	P	47	18	38%
Hussain 2017	India	Broiler and free-range chicken meat	Chicken	P	168	63	38%
LeCuyer 2018	USA	Urinary tract infection	Dog	P	295	14	5%
Liu 2015	USA	Urinary tract infection	Cat	P	2686	76	3%
Liu 2016	China	Naturally occurring infection	Cat, Cattle, Dog, Horse	P	165	40	24%
Liu 2017	China	Urine, blood and feces	Cat, Dog	P	174	16	9%

Table 1. Characteristics of studies included in review (cont'd)							
Liu 2018	China	River and Lake Water	N/A	P	74	8	11%
Maeyama 2018	Japan	Urinary tract infection	Cat, Dog	P	381	78	20%
Nebbia 2014	Italy	Urinary tract infection	Cat	P	138	7	5%
Solà-Ginés 2015	Spain	colibacillosis cases	Chicken	P	32	11	34%
Solà-Ginés 2015	Spain	Broiler farm fly carcass	House Flies	P	682	42	6%
Vounba 2018	Senegal	colibacillosis cases	Chicken	P	58	54	93%
Zogg 2018	Switzerland	Urinary tract infection	Cat, Dog	P	64	35	55%
Zurfluh 2015	Switzerland	Unwashed vegetables	Vegetables	P	169	26	15%

Table 2. Commonly assessed risk factors

	Number of studies investigated (n)	**Number of patients assessed (n)**	**Number of ESBL infections positive for risk factor (n)**	**Number of studies finding significant association (n)**	**% of studies finding significant association (%)**	**% of pooled patients assessed who are ESBL+ (%)**
Previous antibiotic use (previous 3 months)	9	612	159	7	78%	25%
Previous Hospitalization	11	1149	236	8	73%	21%
Gender (Male)	9	1539	232	5	56%	15%
Type II Diabetes	5	526	100	1	20%	19%
Previous UTI	6	131	103	6	100%	79%
Recurrent UTI	5	342	84	5	100%	25%
Catherization	5	80	32	3	60%	40%
Urinary tract abnormality	4	178	45	3	75%	25%


Enviornmental reservoirs of ESBL producing pandemic ExPEC strains
Number of studies
0
2
4
6
8
10
12
ST10
ST69
ST73
ST95
ST127
ST131
Sequence type
vegetables
houseflies
chicken (retail, broiler)
dogs, cats, horse, cattle
water source (rivers, lakes, wastewater)

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CHAPTER TWO: Genetic predictive factors for non-susceptible phenotypes and multidrug resistance in 3rd generation cephalosporin-resistant uropathogenic *E. coli* from a multicenter cohort: insights into the phenotypic and genetic basis of co-resistance

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections in both community and hospital settings, accounting for > 10 million ambulatory visits and incurring $1.6 - 2.8 billion in healthcare costs annually in the US alone (2–5). Moreover, UTIs are the 3rd most common reason for oral antibiotic prescriptions (6). The rising prevalence of multidrug-resistant (MDR) uropathogens increasingly impacts the management of UTIs, with expanded-spectrum cephalosporin resistant Enterobacterales (ESCR-E) causing particular concern (1, 7). The reported prevalence of these organisms among community and hospital-onset UTI isolates is now 15 - 17% (8). ESCR-E have been classified as a serious threat to public health and a critical priority for new antimicrobial development by the Centers for Disease Control and Prevention and the World Health Organization (9, 10). Phenotypic resistance to ESC (3rd generation cephalosporins) is conferred predominantly by extended-spectrum β-lactamases (ESBL) and chromosomal or plasmid-mediated AmpC (cAmpC and pAmpC) β-lactamases. ESBLs hydrolyze penicillins, oxyimino-cephalosporins (ceftazidime, cefotaxime, ceftriaxone, and cefepime), and monobactams (aztreonam), whereas cAmpC and pAmpC enzymes possess the above spectrum of activity, with the exception of cefepime which they cannot hydrolyze (11, 12).

Major UTI syndromes include cystitis, pyelonephritis, and prostatitis, with complicated UTI (cUTI) being defined as those that occur in the setting of immunosuppression or urinary obstruction, carrying a higher risk of treatment failure (13–15). Uncomplicated cystitis is generally treated empirically with oral antibiotics, without obtaining urine culture (16). However, urine culture is strongly recommended in cases of pyelonephritis and cUTI (17). Initial broad-spectrum empiric treatment, typically with an oral fluoroquinolone or parenteral 3rd-generation cephalosporin, can potentially be stepped down based on culture and susceptibility results (14). Empiric treatment that is discordant with susceptibility results occurs more frequently in ESCR than ESC-susceptible UTI, and is associated with prolonged hospitalization and higher mortality (1, 18–20). Treatment of ESCR-E infections is further complicated by the high rates of co-resistance to non-β-lactam antimicrobial agents (fluoroquinolones, trimethoprim-sulfamethoxazole, and aminoglycosides) which limits treatment options and promotes the use of broad-spectrum agents (12, 21). Consequently, carbapenems are increasingly used both for empiric treatment of cUTI and culture-directed treatment of ESCR-UTI (22, 23). This trend is concerning, as carbapenems are considered "last-line" therapy for Gram-negative bacterial infections, and increased use is associated with the emergence of carbapenem-resistant Enterobacterales (CRE) (24–26). To preserve the effectiveness of last-line antibiotics, there have been calls to develop and evaluate carbapenem sparing strategies in ESCR-UTI management (23, 27, 28).

Although ESCR-E has been broadly investigated worldwide, there have been no large-scale multicenter genomic studies of uropathogenic ESCR-E. The overarching aim of this study is to understand the phenotypic and genetic basis of co-resistance within ESCR uropathogenic *E. coli* (UPEC), focusing on the genetic predictors of co-resistance and MDR. This study focused on

UPEC, the most common causative pathogen in UTI, accounting for roughly 70% of all cases (29). Increasing our understanding of genetic predictors for MDR and co-resistance within ESCR UPEC could provide opportunities for improved surveillance and prescribing practices for resistant uropathogens.

RESULTS

Patient demographic characteristics, clinical specimens, and isolate typing information

Five hundred and seventy-seven *E. coli* isolates, resistant to at least one 3rd generation cephalosporin (ESCR UPEC) were included in this analysis. The source of urine specimens included voided urine, (N=269, 46.6%), bladder catheterization, (N=84, 14.6%), or was unknown. Among the study population, 409 (70.9%) of 577 samples originated from females, and patient age ranged from 0 – 102 years old, with the age group 64 – 79 years contributing 173 (30%) samples. Samples were obtained from 6 clinical laboratory sites across California; Site 4 contributed 145 (25.1%) isolates, while the remaining 5 sites contributed 81-106 isolates each. Overall, 527 (91.3%) isolates were phenotypically confirmed to produce an ESBL (defined ESBL phenotype). Male sex was associated with MDR (P=0.0024). Prevalence of ESBL-producing UPECs ranged from 12.9%-26% across locations (P=0.004). We did not have access to the type of UTI syndrome associated with each specimen or the clinical information needed to determine whether UTI was considered a complicated infection.

From genotyping the 577 ESCR UPEC isolates, 7 of the 8 known *E. coli* phylogenetic groups were identified, which included 482 (83.5%) isolates from the ExPEC-associated phylogroups, 356 (61.7%) B2, 75 (13%) D, 51 (8.8%) A, in addition to 95 (16.5%) isolates from other phylogroups. Further characterization of the collection by MLST revealed 73 distinct sequence types, as well as 4 isolates of unknown sequence type. The predominant MLSTs were ST131 (46%), ST1193 (5.5%), ST648 (4.5%), ST69 (4.5%), ST38 (3.8%), ST636 (3.1%) and ST410 (1.7%) (Figure S1).

Antimicrobial susceptibility and multidrug resistance profiles in ESBL and non-ESBL ESCR UPEC

Antimicrobial susceptibility and co-resistance of isolates with ESBL and non-ESBL phenotypes were characterized (Table 2 and Figure 1). The frequency of antimicrobial non-susceptibility differed between ESBL phenotype vs. non-ESBL phenotype ESCR UPEC, including non-susceptibility to ciprofloxacin (82% vs. 40%, P=<0.001), levofloxacin (84% vs. 40%, P=<0.001), tobramycin (49% vs. 22%, P=0.004), cefepime (51% vs. 8%, P=<0.001), and to cefotaxime (99.6% vs. 78%, P=<0.001); Figure 1 and Table S1. Antimicrobial non-suceptibility, ESBL phenotype and MDR stratified by common MLSTs is shown in Table S2.

Overall, 388 (67%) isolates were MDR (Figure 1). MDR prevalence was 1.7 times greater in ESBL phenotype vs. non-ESBL phenotype UPEC (69.8% vs. 40%, P=<0.001); Table S1. MDR prevalence also varied across predominant MLSTs, ranging from 0% in ST636, to 81.6% in ST131 (Table S2). We went on to further characterize the resistance profiles of MDR isolates. Resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, in addition to β-

lactams, was found in 150 (39%) isolates classified as MDR. Antimicrobial resistance profiles stratified by ESBL phenotype are shown in Table S3.

Whole-genome sequence analysis for identification of resistance mechanisms and replicon types in ESCR UPEC

β-lactamase genes, horizontally acquired resistance genes, and mutations known to confer antimicrobial resistance were characterized within the 577 ESCR UPEC. Genes encoding β-lactamases known to confer resistance to ESCs were identified in 564 (97.7%) of the ESCR UPEC isolates. blaCTX-M ESBL genes were present in 530 (91.8%) isolates, whereas blaCMY pAmpC genes were present in 48 (8.3%) isolates. The carbapenemase variant, blaKPC-2, was detected in one isolate. Narrow-spectrum (non-ESBL) blaTEM genes were detected in 207 (44.9%) isolates, whereas narrow-spectrum (non-ESBL) blaOXA genes, were identified in 184 (32%) isolates. In 173 (29.9%) isolates, both blaOXA-1 and blaCTX-M-15 genes were identified. The prevalence of certain β-lactamase genes differed between ESCR UPEC with ESBL and non-ESBL phenotypes, including variants of the blaCTX-M (99.4% vs. 12%, P=<0.001), blaCMY (2.9% vs. 66%, P=<0.001) and blaOXA (34.4% vs. 8.0%, P=0.004) respectively (Figure 3 and Table S4).

Overall, 526 (99.8%) of ESBL phenotype vs. 7 (14.0%) of non-ESBL phenotype isolates carried an ESBL gene, while 17 (3.2%) of ESBL phenotype vs. 31 (62.0%) of non-ESBL phenotype isolates carried a pAmpC gene. Fourteen (2.3%) ESCR UPEC isolates contained both ESBL and pAmpC β-lactamase genes; of these, 13 (92.8%) had an ESBL phenotype vs. 1 (7.1%) with a non-ESBL phenotype. Thirteen (2.3%) ESCR UPEC contained no characterized β-lactamase genes which confer resistance to 3rd-generation cephalosporins; within these isolates, 5 (38.5%) contained cAmpC promoter mutations, 4 (30.8%) contained a blaTEM-1B gene, 1 (7.7%) contained a blaOXA-1 gene, 1 (7.7%) contained a blaCARB-2 gene, and 7 (53.8%) isolates contained no characterized β-lactamase genes.

Horizontally acquired genes which confer resistance to 8 distinct antimicrobial classes (other than β-lactams) were identified, including genes known to provide protection against agents commonly used to treat UTI (trimethoprim-sulfamethoxazole, fluoroquinolones, and aminoglycosides), and genes conferring resistance to polymyxins, tetracyclines, macrolides and florfenicol (Figure 2). Several resistance genes differed in prevalence between ESBL and non-ESBL UPEC: aac(6')-Ib-cr (34.5% vs. 8.0%, P=0.004) and aac(3)-IIa (26.0% vs. 4.0%, P=0.026), which confer resistance to aminoglycosides, Table S5.

Mutations which are known to confer resistance to fluoroquinolones, polymyxins, and tetracyclines were also identified (Table S4). Regarding fluoroquinolone resistance, mutations in the gene encoding DNA gyrase subunit A were identified in 459 (79.5%) isolates, whereas mutations in topoisomerase IV genes were detected in 424 (73.5%) isolates. The proportion of DNA gyrase mutations, gyrA (S83L) (83.7% vs. 36%, P=<0.001) and gyrA (D87N) (68.5% vs. 32%, P=<0.001) and the topoisomerase mutations, parC (S80I) (72.5% vs. 34.0%, P=<0.001), parC (E84V) (43.5% vs. 10.0%, P=<0.001) and parE (I529L) (46.9% vs. 12.0%, P=<0.001) differed between ESBL phenotype and non-ESBL phenotype isolates, respectively (Table S5).

Regarding polymyxin resistance, a pmrA gene mutation (R81S) was identified in 1 (0.2%) isolate, whereas a mutation in the 16s rRNA operon, rrsB (G1058C) conferring resistance to tetracyclines was identified in a single isolate (0.2%) also (Table S5).

Regarding plasmid content, 16 distinct replicon types were identified with IncFIB and IncFII detected in 435 (75.4%) and 410 (71.1%) isolates, respectively. Furthermore, the prevalence of replicons between isolates with ESBL and non-ESBL phenotypes was different for IncFIA (61.3% vs. 38%, P=0.041), and IncHI (10.8% vs. 34%, P=<0.001) (Figure 2 and Table S6).

Analysis of antimicrobial resistance genes, and determination of positive predictive value between genotypes and non-susceptible phenotypes within ESCR UPEC

We investigated the probability of a non-susceptible phenotype given the presence of related and unrelated genotypes, by estimating the positive predictive value (PPV) (Figure 4 and Table S7). The most common classes of horizontally-acquired resistance genes that were identified from the WGS analysis were included in the analysis (prevalence ≥ 5%), alongside the susceptibility data for agents used to treat UTI, including the fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (tobramycin, gentamicin, amikacin), β-lactams (ampicillin-sulbactam, piperacillin-tazobactam, cefepime, ertapenem), nitrofurantoin, and trimethoprim-sulfamethoxazole. We also carried out a gene-gene correlation analysis using the Phi correlation coefficient for all ESCR UPEC strains (N=577) and for those with an ESBL phenotype (N=527) (Figure S2).

The probability of non-susceptible phenotypes given the presence of a recognized horizontally acquired resistance gene, ranged from 0 - 1. When examining non-susceptibility to fluoroquinolones, the presence of blaCTX-M, blaTEM, sul, dfrA/B, aad, ant and aph were associated with PPVs that ranged from 0.7 - 0.89, whereas the presence of blaOXA or aac(6')-Ib-cr was associated with a PPV ≥ 0.9. The genes blaOXA and aac(6')-Ib-cr were found to be strongly correlated with one another ($\Phi = 0.9$), whereas blaCTX-M-15 was moderately correlated ($\Phi = 0.5$) with both blaOXA and aac(6')-Ib-cr. Hierarchical cluster analysis of PPV reveals a strong clustering of blaOXA and aac(6')-Ib-cr. The genes dfrA/B, aad and ant demonstrated a PPV of non-susceptibility to trimethoprim-sulfamethoxazole of ≥ 0.9. The genes, sul, blaTEM, qnrB, qnrS and aph displayed PPVs ranging from 0.7 - 0.89 for non-susceptibility to trimethoprim-sulfamethoxazole. The results of the gene correlation analysis displayed a moderate to strong positive correlation ($\Phi = 0.4 – 0.8$) between trimethoprim-sulfamethoxazole resistance determinants, sul and dfrA/B, with the aminoglyoside resistance determinants, aad, ant and aph, and a weak positive correlation ($\Phi = 0.2$) with blaTEM genes. The genes blaOXA, aac(6')-Ib-cr, and aac each respectively had a PPV for non-susceptibility to tobramycin ≥ 0.9, and aac had a PPV ≥ 0.9 for gentamicin non-susceptibility. In addition to the strong correlation with aac(6')-Ib-cr ($\Phi = 0.9$), blaOXA had a moderate correlation with other aac genes ($\Phi = 0.4$). None of the genes included in this analysis were predictors for amikacin or nitrofurantoin non-susceptibility (Figure 4 and Table S7).

Antibiotic resistance genes as predictors of antibiotic non-susceptibility and MDR within ESCR UPEC

We assessed the presence of common β-lactamase genes and other resistance genes as predictors of resistance to agents used to treat UTI. From the correlation and PPV analyses, an association between blaCTX-M-15, blaOXA-1 and aac(6')-Ib-cr genes was identified. Therefore, we first calculated the risk ratio of the co-occurrence of these genes and antibiotic non-susceptibility (MIC indicating intermediate susceptibility or resistance). The co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-Ib-cr (found in 32.1% of ESBL phenotype isolates) increased the risk of non-susceptibility to all agents included in the analysis, with the exception of nitrofurantoin and trimethoprim-sulfamethoxazole. The presence of these genes led to an 8-fold increase in risk of non-susceptibility to amikacin (RR=8), an aminoglycoside used in the empiric treatment of cUTI, and the treatment of clinically confirmed ESCR UPEC UTIs. The co-occurrence of these genes also resulted in a 1.79-fold increase in risk of MDR (RR=1.79) (Table 3).

To examine the individual risk of non-susceptibility associated with the presence of individual genes while controlling for the effect of sequence type, we conducted logistic regression. We defined the primary outcome of these models to be non-susceptibility to antibiotics used in the treatment of UTI. We also included MDR as an outcome (Figure 5 and Table S8). Our predictors of interest were the most prevalent blaCTX-M variants identified, the most common pAmpC identified (blaCMY-2), and the narrow spectrum β-lactamase genes, blaTEM-1B and blaOXA-1. We controlled for confounding by sequence type by including ST131 classification in our model, as it was the most dominant MDR associated sequence type (46% of isolates). We also included aac(6')-Ib-cr in our model, due to the previously identified association of this gene with blaOXA-1 and blaCTX-M-15. Results indicate that the risk of antibiotic non-susceptibility differs between the presence of blaCTX-M variants and blaCMY-2 (piperacillin-tazobactam, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, and cefepime), in addition to between blaCTX-M variants (ampicillin-sulbactam, fluoroquinolones, gentamicin, tobramycin and cefepime). With the exception of blaCTX-M-55 (OR 3.7; 95% CI 1.5 – 9.4), the presence of blaCTX-M or blaCMY-2 variants alone were not predictors of MDR, and instead, blaTEM-1B (OR 2.0; 95% CI 1.3 – 3.5) aac(6')-Ib-cr (OR 33.8; 95% CI 4.9 – 313) or the ST131 genotype (OR 2.2; 95% CI 1.4 – 3.7) were identified as predictors of MDR within ESCR UPEC. The only predictor of non-susceptibility to the carbapenem sparing agent, piperacillin-tazobactam, was blaCMY-2 (OR 4.9; 95% CI 1.3 – 20.4). Detailed results of the regression analysis can be found in the supplementary information, Table S8.

An additional regression was carried out, examining the association between the most prevalent gyrase and/or topoisomerase IV mutations (gyrA: D87N, S83L and E84V; parC: E84V and S80I; parE: I529L, L416F and S458A) with fluoroquinolone non-susceptibility, controlling for the effects of resistance genes described previously, in addition to the lineage, ST131 (Table S9). The results indicate that the ST131 lineage (OR 3.06, 95% CI 1.49 – 6.61, P=0.003), aac(6')-Ib-cr (OR 56.48, 95% CI 4.08 – 865.12, P=0.003) and prevalent gyrase and/or topoisomerase IV mutations (OR 16.42, 95% CI 8.84 – 31.77, P=<0.001) were predictors for fluoroquinolone non-susceptibility. No β-lactamase genes were identified as predictors for fluoroquinolone non-

susceptibility, however blaTEM-1B had a protective effect (OR 0.36, 95% CI 0.19 – 0.66, P=0.001).

Pan-genome and phylogenetic analysis

WGS information for the ESCR UPEC isolates was subjected to pan-genome and phylogenetic analysis (Figure 6). A total of 32,586 genes constituted the pan-genome. Of these, 2,967 (9%) were shared among more than 95% of the isolates (core genes) and 29,619 (91%) were distributed among subsets of the isolates (accessory genes). Of the latter, 26,437 genes were found in <15% of the isolates (cloud genes).

Maximum-likelihood phylogenies of the SNP alignment of core genomes and the presence and absence of accessory genomes were obtained from FastTree (9) with the Jukes-Cantor model (Figure 6). To assess the distribution of genes of interest, the most prevalent sequence types were highlighted, alongside fimH type and the dominant β-lactamase genes conferring a ESCR phenotype (blaCTX-M-15, blaCMY-2). Genes previously identified as risk factors for MDR were also highlighted, including blaCTX-M-55, blaTEM-1B, and the co-occurrence of blaOXA-1 and aac(6')-Ib-cr with blaCTX-M-15.

Clustering based on sequence type and clonal group was observed; for instance, in the ST131 clonal group, ST131, ST2279, ST8257, and ST8671 were present in a single cluster. Clustering based on fimH types was observed within ST131 phylogenetics, with fimH30 predominating. Regarding resistance genes, clustering based on the carriage of different classes of β-lactamase genes was observed in all sequence types. Isolates with a co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-lb-cr genes and isolates with only blaCTX-M-15 belong to different clades, a trend which is observed in all sequence types. The results of this analysis also highlight that the majority of isolates with a co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-lb-cr genes are associated with the ST131 lineage, as 76.5% of all isolates in which these three genes were detected were ST131. This co-occurrence was also observed in the emerging lineages, ST1193 and ST648, the MDR lineage, ST410, as well as 12 other characterized MLSTs, and in isolates of unknown MLST. Isolates containing blaCTX-M type genes and blaCMY-2 also belonged to different clades. The blaCTX-M-55 gene was identified within 20 distinct MLSTs, with the emerging lineage, ST1193 and ST774 harboring 14.6% and 16.7% respectively, of all blaCTX-M-55 genes detected. The blaTEM-1B gene was also present in 35 (47.9%) of 73 characterized lineages identified in this collection.

DISCUSSION

The primary aim of this study was to characterize the phenotypic antimicrobial susceptibility and underlying genotypes of ESCR UPEC isolated from patients with UTI in California, in addition to identifying genetic risk factors for non-susceptibility and MDR. We observed a predominance of high-risk, MDR-associated ExPEC lineages ST131 (46%), ST69 (4.5%) and ST410 (1.7%), as well as emerging MDR lineages ST1193 and ST648 (collectively, 10% of isolates) (30–33). We also describe the frequency of β-lactamase genes, which underly the ESCR phenotype. As expected, 3rd generation cephalosporin resistance was predominantly attributed to the presence of either ESBL or pAmpC β-lactamases and within these two groups, the blaCTX-M-15 and

blaCMY-2 genes were the most prevalent, respectively. This is unsurprising, as blaCTX-M-15 and blaCMY-2 genes predominate globally in ESCR-E. However, the emerging ESBL variants, blaCTX-M-27 and blaCTX-M-55, were also identified in a notable proportion (18.3% and 9.1%, respectively) of isolates (34–38). The correlation analysis also highlighted an inverse correlation of the co-occurrence of ESBL and pAmpC type β-lactamase genes, meaning that within this data set, the co-occurrence of these genes was unlikely; as a result, only 14 (2.3%) isolates contained both ESBL and pAmpC genes, and of these isolates, 13 (92.8%) retained an ESBL phenotype. This suggests that phenotypic ESBL testing is (for the most part) reliable, as the potential co-occurrence of pAmpC and ESBL β-lactamases, which has been cited as a reason against the further use of this test, is rare at least within the study region of California (39).

Our results suggest that identifying the ESBL phenotype (by rapid diagnostics, for instance) in clinical practice has the potential to significantly reduce unnecessary UTI treatment escalation. In current practice, when ESCR is confirmed, treatment is often escalated to a carbapenem due to the association of ESBL with MDR (40, 41). However, ESBL confirmatory testing of ESCR isolates is no longer advised by the CLSI, and no standardized approaches have been established for the phenotypic confirmation of pAmpC β-lactamases (42, 43). In our study population, large differences are seen between ESBL phenotype and non-ESBL phenotype ESCR UPEC in their susceptibility to agents commonly used to treat UTI. In fact, one-third of ESCR isolates in this study, which would have been classified as MDR organisms remained susceptible to at least one narrow-spectrum agent. This represents a large potential opportunity to spare the use of broad-spectrum antibiotics, particularly carbapenems. This potential increases further if ESBL phenotype status is known, as the prevalence of MDR organisms among ESBLs was 1.7 times higher than non-ESBL organisms (P=<0.001). One limitation of this study is that by restricting to ESCR organisms, examining risk factors for MDR is subsequently not generalizable to all UPEC infections. However, these risk factors still provide the opportunity to reduce unnecessary treatment escalation for patients who screen positive for ESBL.

In the identification of genetic predictive factors for non-susceptibility, the emerging ESBL variant, blaCTX-M-55 and the lineage ST131 were identified as strong predictors of MDR within ESCR UPEC. ST131 is recognized as a problematic MDR lineage globally, whereas MDR Enterobacterales harboring blaCTX-M-55 have been previously identified from clinical samples in China and France, in addition to poultry samples from Brazil (44, 45, 38, 46–48). The genotypic and phylogenetic analysis carried out in this study has revealed a strong correlation between blaCTX-M-15, blaOXA-1 and aac(6')-Ib-cr among the ESCR UPEC. These genes were commonly found together and were present in almost one-third (N=169) of all ESBL phenotype isolates. This association has been identified previously in ESBL producing *E. coli* isolates from the UK and Portugal, and was shown to correlate with resistance to piperacillin-tazobactam, co-amoxiclav, and tobramycin (49, 50).

This research highlights the co-occurrence of blaCTX-M-15, blaOXA-1 and aac(6')-Ib-cr within ESCR UPEC as a risk factor for MDR, as well as for non-susceptibility to agents used to treat UTI, including carbapenem-sparing agents (piperacillin-tazobactam, amikacin and the fluoroquinolones). In fact, the co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-Ib-cr was a

strong risk factor for non-susceptibility to amikacin. The gene, aac(6')-Ib-cr, encodes a bi-functional aminoglycoside modifying acetyltransferase, which has been shown to confer resistance to tobramycin, kanamycin and amikacin, in addition to the fluoroquinolones (51). This variant is characterized by the presence of two amino acid changes, at codons 102 (Trp→Arg) and 179 (Asp→Tyr) when in comparison its predecessor, aac(6')-Ib, with these mutations conferring an extended spectrum of activity towards fluoroquinolones (52). Despite this, the results of the regression analysis showed that the presence of aac(6')-Ib-cr gene alone was not sufficient to confer resistance to amikacin, suggesting that other genetic mechanisms (efflux, 16s rRNA methylation or interplay with other horizontally acquired aminoglycoside resistance mechanisms (53) may contribute to amikacin resistance in isolates co-harboring blaCTX-M-15/blaOXA-1/aac(6')-Ib-cr; further investigation is needed to understand the mechanistic basis of amikacin non-susceptibility in these isolates. However, aac(6')-Ib-cr did increase the risk of non-susceptibility to fluoroquinolone antibiotics, highlighted from the results of the regression analysis in which we controlled for confounding by prevalent gyrase and/or topoisomerase IV mutations. However, previous studies into the mechanistic basis of aac(6')-Ib-cr have shown that this gene alone cannot confer clinical levels of resistance to fluoroquinolones, therefore there may be interplay between aac(6')-Ib-cr and other fluoroquinolone resistance mechanisms within isolates from this collection which were not controlled for in this analysis (54); further investigation would be required to understand the contribution of aac(6')-Ib-cr to fluoroquinolone non-susceptibility within the isolates from this study. Lastly, the presence of blaOXA-1 in ESBL producers has been recognized previously to reduce susceptibility to penicillin-inhibitor combinations. It has been postulated that ESBL isolates co-expressing blaOXA-1 were responsible for the inferiority of piperacillin-tazobactam, as compared to carbapenems, in the treatment of ESCR infections in the MERINO trial (55).

In conclusion, this study provides a regional description of prevalent MDR UPEC lineages, phenotypic co-resistance profiles, and resistance determinants in uropathogenic ESCR UPEC. This study highlights targets for improved antimicrobial resistance surveillance and helps identify putative genes underlying the ESCR phenotype within clinical isolates in California. Elucidating the specific β-lactamase genes present in these suspected MDR organisms increases our understanding of associated co-resistance profiles. Our results suggest the co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-Ib-cr genes and the occurrence of blaCTX-M-55, constitutes an important risk factor for MDR in ESCR UPEC. Identification of these markers, in addition to ESBL phenotype could inform empiric treatment decisions, including targeted carbapenem sparing strategies, for ESCR UTIs, to not only promote antibiotic stewardship, but improve treatment outcomes. Antimicrobial resistance is now recognized as a leading cause of mortality worldwide (56), and therefore, sustained efforts must be made to curb unnecessary antibiotic use through improved prescribing practices, to alleviate the public health burden of drug-resistant infections.

MATERIALS AND METHODS

Clinical samples and isolate collection

Between February and October of 2019, we collected *E. coli* isolated from urine specimens (UPEC), from six different clinical laboratory sites across California (4 sites in northern and 2 sites in southern California). Only *E. coli* growing at clinically significant thresholds as determined per standard operating procedures at each site, and isolates which tested non-susceptible (intermediate or resistant MIC value) to 3rd generation cephalosporins (cefotaxime, ceftazidime, or ceftriaxone; ESCR) according to CLSI interpretive criteria (40), were selected for further analysis (N=577 isolates). Duplicate patient samples identified were removed from the analysis.

Susceptibility testing and ESBL confirmatory testing

Susceptibility testing was conducted by the respective clinical laboratories from which isolates were collected; minimum inhibitory concentrations (MICs) were determined in accordance with CLSI guidelines, and were performed on the MicroScan WalkAway (Neg/Urine Combo type 73 panel; Beckman Coulter, Brea, CA, USA), Trek Sensititre (GN6F panel; Thermo Scientific, Emeryville, CA, USA) or VITEK 2 (AST-GN90 panel; bioMérieux, Inc, Durham, NC, USA). MIC results were interpreted as susceptible, intermediate, or resistant according to interpretative criteria outlined in current CLSI guidelines (40). Susceptibilities to the following antimicrobial agents were included in this study, due to their importance in the treatment of UTI: cefotaxime, ceftazidime, ceftriaxone, cefepime, ampicillin-sulbactam, piperacillin-tazobactam, ertapenem, amikacin, tobramycin, gentamicin, ciprofloxacin, levofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole. If susceptibility information was missing for a given isolate, susceptibility testing was carried out using the disk diffusion method, in accordance with CLSI guidelines (40). An MDR isolate was defined as one that tested resistant to at least 1 agent in ≥ 3 classes of antimicrobial agents included in this analysis: β-lactams, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole and nitrofurantoin.

ESBL confirmatory testing was performed with the disk diffusion method (using disks containing cefotaxime, cefotaxime + clavulanic acid, ceftazidime, and ceftazidime + clavulanic acid) per CLSI guidelines (40). For quality control, the CLSI recommended reference strains, ATCC 25922 and ATCC 700603, were tested alongside clinical isolates (40).

Whole genome sequencing and genome assembly

DNA was extracted from *E. coli* isolates and the libraries were prepared with the Nextera DNA Flex Library Prep Kit (Illumina, USA), before libraries were sequenced on the MiSeq platform (Illumina, USA). MiSeq reads were screened and trimmed based on length and quality with BBDUK (version 1.0) under the default setting (57). The trimming process also removed residual adapter sequences. Quality check of individual FASTQ files was conducted with FastQC (58). De novo assembly of trimmed paired reads for the libraries was performed with Unicycler (version 0.4.8) under the setting "--min_fasta_length 500" to remove contigs less than 500bp (59). The number of reads used in each assembly was sufficient to give a minimum of 25-fold coverage, averaged across all contigs. A maximum of 45-fold coverage was obtained. Contigs that were less

than 500bp in length and with less than 80% high-quality base calls were eliminated from subsequent analysis.

Annotation was performed on all the assembled genomes with Prokka (version 1.14.0) (60). Full assemblies were uploaded to the Batch Processing portal at Center for Genomic Epidemiology to confirm species, identify plasmid replicons, antibiotic resistance, and virulence genes (61). fimH types were identified with the FimTyper database (accessed April 2020) (62). All identifications were done with Abricate (version 1.0.1) (https://github.com/tseemann/abricate) with a 95% identity threshold across the reference sequences. Multilocus sequence typing was determined with mlst (version 2.19.0) (https://github.com/tseemann/mlst) based on the seven-gene Achtman scheme (63, 64).

In the description of the β-lactamase genes identified from our WGS analysis, we defined β-lactamases other than ESBL, pAmpC and carbapenemases (such as non-ESBL TEM, SHV and OXA variants) as having a 'narrow-spectrum' of activity.

Bioinformatics and phylogenetic analysis

A pangenome of all de novo assemblies (N=577) and of ST131 (N= 267) was constructed with Roary (version 3.13.0) with a 95% identity cutoff (65). Here, genes present in ≥95% of the cohort isolates were defined as core and constituting the core portion of the metagenome, and genes present in less than 95% were defined as accessory and constituting the accessory portion of the metagenome. Accessory genes present in <15% of the cohort isolates were defined as cloud genes. A concatenated core CDS alignment was made from the Roary output, and we extracted single nucleotide polymorphism (SNP) information with SNP-sites with the default option (66). Phylogenetic trees were constructed with FastTree (version 2.1.10) with the maximum likelihood method with the Jukes-Cantor model based on the SNP alignment, and the presence and absence of accessory genes from the Roary output (67). Visualization was done with iToL, version 6.1.2 (http://itol.embl.de).

Statistical analysis and data visualization

Data visualization, generation of graphs and statistics were performed with R 3.0.1. Adjustment for multiple comparisons was done using the Bonferroni method (68).

(https://www.r-project.org/). The R packages and functions used in this study included, ggplot2 (https://www.rdocumentation.org/packages/ggplot2/versions/3.3.5), corrplot (https://www.rdocumentation.org/packages/corrplot/versions/0.2-0/topics/corrplot), heatmap (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/heatmap) chordDiagram (https://www.rdocumentation.org/packages/circlize/versions/0.4.13/topics/chordDiagram)

glm (https://www.rdocumentation.org/packages/SparkR/versions/2.1.2/topics/glm), and

VennDiagram (https://cran.r-project.org/web/packages/VennDiagram/VennDiagram.pdf). Figures were resized or re-labelled on Adobe Illustrator (version 25.0.01) (Adobe Systems Incorporated, San Jose, CA, USA)

Patient characteristics	Non-MDR (N=189)	MDR (N=388)	Non-ESBL (N=50)	ESBL (N=527)	Overall (N=577)
Sex (Male)	43 (22.8%)	125 (32.2%)	10 (20%)	158 (30%)	168 (29.1%)
Age					
0-17	6 (3.2%)	10 (2.6%)	2 (4.0%)	14 (2.7%)	16 (2.8%)
18-44	46 (24.3%)	59 (15.2%)	14 (28.0%)	91 (17.3%)	105 (18.2%)
45-63	51 (27.0%)	109 (28.1%)	8 (16.0%)	152 (28.8%)	160 (27.7%)
64-79	55 (29.1%)	118 (30.4%)	16 (32.0%)	157 (29.8%)	173 (30.0%)
80+	31 (16.4%)	92 (23.7%)	10 (20.0%)	113 (21.4%)	123 (21.3%)
Specimen					
Catheter	26 (13.8%)	58 (14.9%)	5 (10.0%)	79 (15.0%)	84 (14.6%)
Voided	93 (49.2%)	176 (45.4%)	33 (66.0%)	236 (44.8%)	269 (46.6%)
Surgical	3 (1.6%)	6 (1.5%)	1 (2.0%)	8 (1.5%)	9 (1.6%)
Suprapubic aspirate	1 (0.5%)	1 (0.3%)	0 (0%)	2 (0.4%)	2 (0.3%)
Unknown	66 (34.9%)	147 (37.9%)	11 (22.0%)	202 (38.3%)	213 (36.9%)
Location					
Site 1	27 (14.3%)	53 (13.7%)	12 (24%)	68 (12.9%)	80 (13.9%)
Site 2	26 (13.8%)	64 (16.5%)	13 (26%)	77 (14.6%)	90 (15.6%)
Site 3	20 (10.6%)	61 (15.7%)	1 (2%)	80 (15.2%)	81 (14%)
Site 4	48 (25.4%)	97 (25%)	8 (16%)	137 (26%)	145 (25.1%)
Site 5	29 (15.3%)	46 (11.9%)	4 (8%)	71 (13.5%)	75 (13%)
Site 6	39 (21%)	67 (17.3%)	12 (24%)	94 (17.8%)	106 (8.4%)

Table 1. Patient characteristics and distribution by site of ESCR UPEC, stratified by MDR and ESBL phenotype. An MDR isolate was defined by phenotypic resistance to at least 1 agent in ≥ 3 classes of antimicrobial agents used to treat UTI (β-lactams, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole and nitrofurantoin). An ESBL phenotype was defined by confirmation of ESBL production by the disk diffusion method of ESBL confirmatory testing.

Antimicrobial agent	Non-ESBL (%) [N=50]			ESBL (%) [N=527]			Total (%) [N=577]		
	S	I	R	S	I	R	S	I	R
β-lactams or β-lactams/β-lactamase inhibitor combinations									
Ceftazidime	8 (16.0%)	15 (30.0%)	27 (54.0%)	150 (28.5%)	132 (25.0%)	245 (46.5%)	158 (27.4%)	147 (25.5%)	272 (47.1%)
Ceftriaxone	1 (2.0%)	4 (8.0%)	45 (90.0%)	2 (0.4%)	0 (0%)	525 (99.6%)	3 (0.5%)	4 (0.7%)	570 (98.8%)
Cefotaxime	11 (22.0%)	3 (6.0%)	36 (72.0%)	2 (0.4%)	3 (0.6%)	522 (99.1%)	13 (2.3%)	6 (1.0%)	558 (96.7%)
Cefepime*	41 (82.0%)	5 (10.0%)*	4 (8.0%)	187 (35.5%)	71 (13.5%)	269 (51.0%)	228 (39.5%)	76 (13.2%)	273 (47.3%)
Ampicillin-sulbactam	6 (12.0%)	13 (26.0%)	31 (62.0%)	161 (30.6%)	140 (26.6%)	226 (42.9%)	167 (28.9%)	153 (26.5%)	257 (44.5%)
Piperacillin-tazobactam	41 (82.0%)	5 (10.0%)	4 (8.0%)	496 (94.1%)	19 (3.6%)	12 (2.3%)	537 (93.1%)	24 (4.2%)	16 (2.8%)
Ertapenem	49 (98.0%)	1 (2.0%)	0 (0%)	525 (99.6%)	0 (0%)	2 (0.4%)	574 (99.5%)	1 (0.2%)	2 (0.3%)
Aminoglycosides									
Amikacin	50 (100%)	0 (0%)	0 (0%)	508 (96.4%)	12 (2.3%)	7 (1.3%)	558 (96.7%)	12 (2.1%)	7 (1.2%)
Gentamicin	39 (78.0%)	0 (0%)	11 (22.0%)	330 (62.6%)	4 (0.8%)	193 (36.6%)	369 (64.0%)	4 (0.7%)	204 (35.4%)
Tobramycin	39 (78.0%)	5 (10.0%)	6 (12.0%)	269 (51.0%)	65 (12.3%)	193 (36.6%)	308 (53.4%)	70 (12.1%)	199 (34.5%)
Fluoroquinolones									
Ciprofloxacin	30 (60.0%)	0 (0%)	20 (40.0%)	96 (18.2%)	14 (2.7%)	417 (79.1%)	126 (21.8%)	14 (2.4%)	437 (75.7%)
Levofloxacin	30 (60.0%)	0 (0%)	20 (40.0%)	83 (15.7%)	46 (8.7%)	398 (75.5%)	113 (19.6%)	46 (8.0%)	418 (72.4%)
Other									
Trimethoprim-sulfamethoxazole	28 (56.0%)	0 (0%)	22 (44.0%)	199 (37.8%)	0 (0%)	328 (62.2%)	227 (39.3%)	0 (0%)	350 (60.7%)
Nitrofurantoin	40 (80.0%)	1 (2.0%)	9 (18.0%)	459 (87.1%)	40 (7.6%)	28 (5.3%)	499 (86.5%)	41 (7.1%)	37 (6.4%)

Table 2: ESCR UPEC susceptibility, stratified by ESBL phenotype. Phenotypic ESBL status and susceptibilities were determined in accordance with CLSI standards (40): Susceptible (S), Intermediate (I), Resistant (R). *In 2016 the intermediate breakpoint for cefepime was changed to susceptible dose-dependent (SDD), as (dependent on the MIC) isolates may remain susceptible to cefepime if the drug dose or frequency of administration is increased. As treatment of SDD organisms relies on accurate MIC determination (therefore does not impact empiric therapies), for the purpose of this study we treated SDD isolates as intermediate or "non-susceptible".

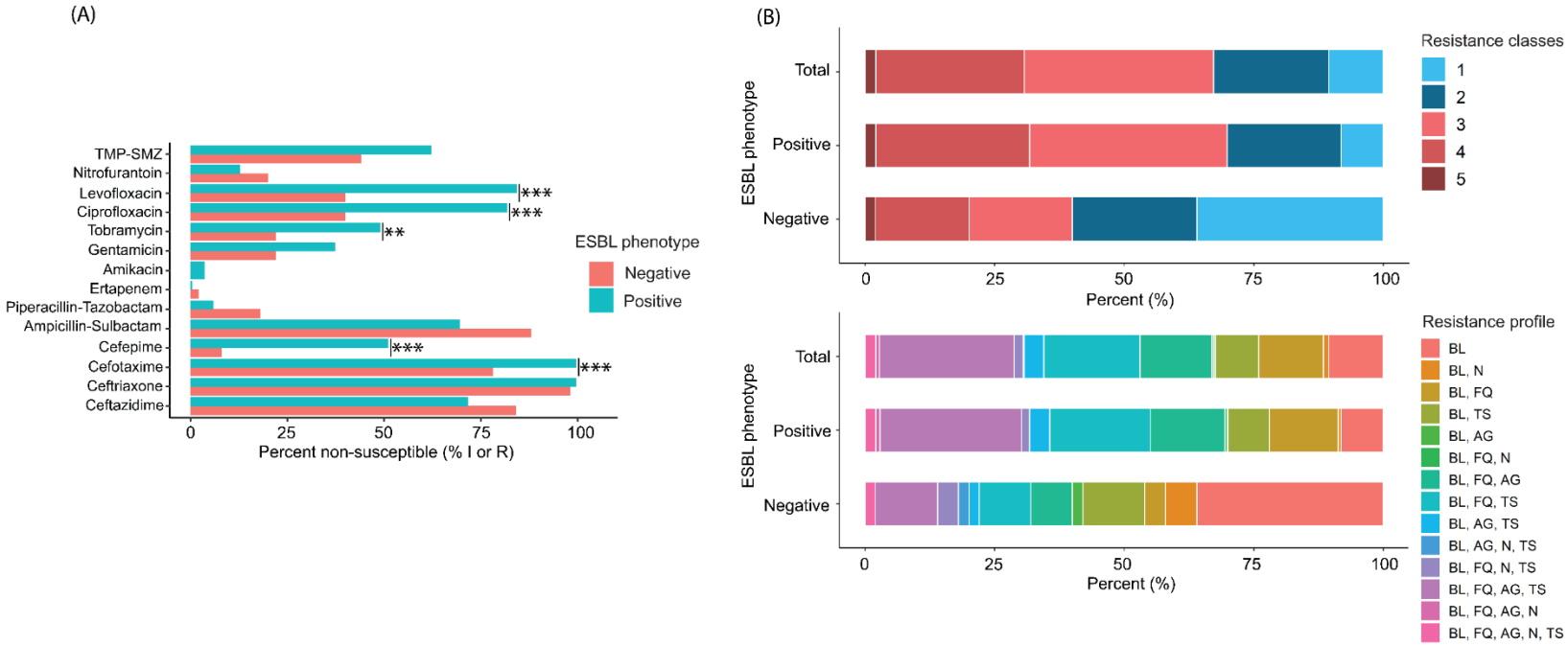
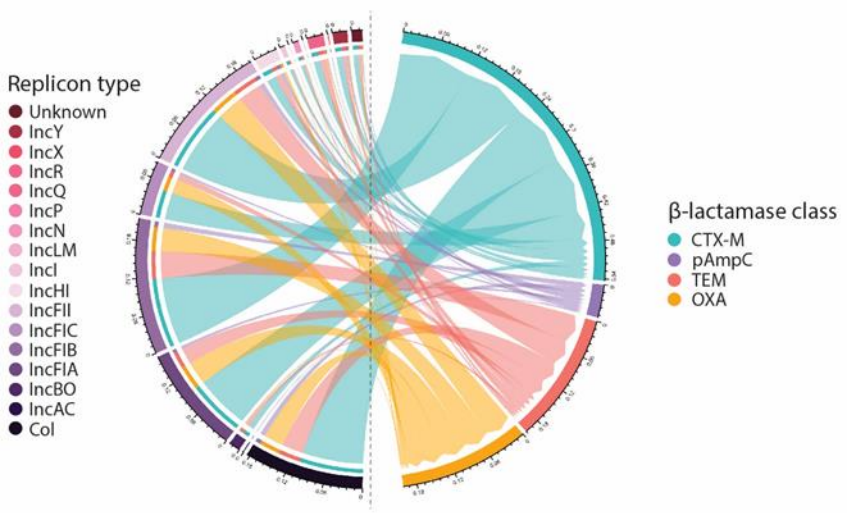
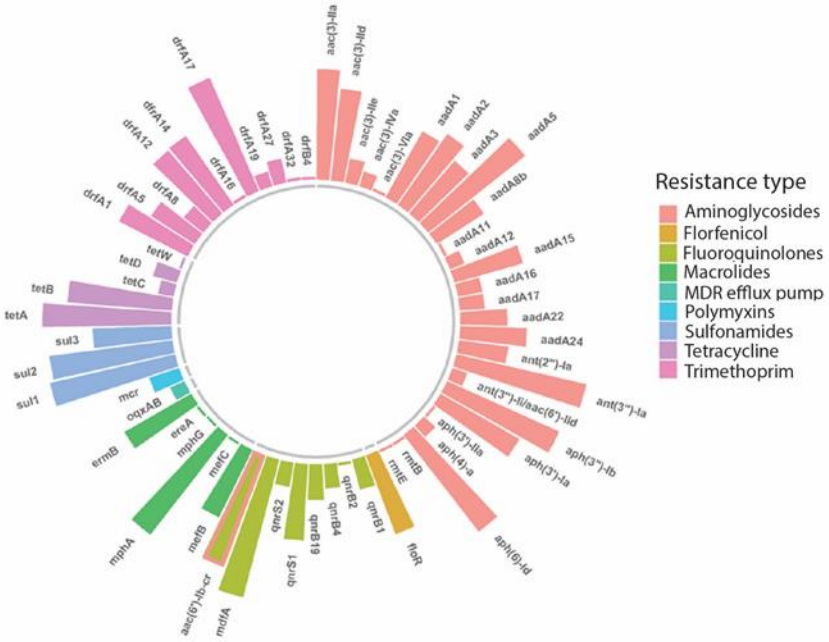


Figure 1: Antimicrobial resistance and MDR profiles in ESCR UPEC (N=577), stratified by ESBL phenotype. (A) Antimicrobial non-susceptibility of ESBL and non-ESBL in ESCR UPEC. Isolates interpreted as intermediate or resistant (I or R) susceptibility according to CLSI breakpoints, were categorized as "non-susceptible". (B) Stacked bar charts displaying prevalence of MDR and characterization of resistance profiles among ESBL and non-ESBL ESCR UPEC. 'Resistance classes' describes the number of distinct antimicrobial classes that isolates were resistant to. Abbreviations are defined as follows: BL = β-lactam, N = nitrofurantoin, FQ = fluoroquinolones, AG = aminoglycosides, TS = trimethoprim-sulfamethoxazole. Statistical analyses were performed using Fisher's exact test, and results were plotted using the ggplot2 function in R 3.0.1. Susceptibility data for all ESCR UPEC (N=577) were included in this analysis. P-value: <0.05 (*), <0.01 (**), <0.001 (***).

(A)


Replicon type
Unknown
IncY
IncX
IncR
IncQ
IncP
IncN
IncLM
IncI
IncHI
IncFII
IncFIC
IncFIB
IncFIA
IncBO
IncAC
Col
β-lactamase class
CTX-M
pAmpC
TEM
OXA

(B)


Resistance type
Aminoglycosides
Florfenicol
Fluoroquinolones
Macrolides
MDR efflux pump
Polymyxins
Sulfonamides
Tetracycline
Trimethoprim
ant(3")-Ia
aph(3")-Ib
aph(6)-Id
floR
qnrS1
qnrB19
qnrB4
qnrB2
qnrB1
qnrS2
mdtA
aac(6')-Ib-cr
metB
mphA
mdtC
mphG
ermB
ereA
oqxAB
mcr
sul1
sul2
sul3
tetA
tetB
tetC
tetD
tetW
drfA1
drfA5
drfA8
drfA16
drfA12
drfA14
drfA17
drfA19
drfA27
drfA32
drfB4
aph(3')-Ia
aph(4)-a
aph(3')-IIa
ant(3")-II/aac(6')-IId
ant(2")-Ia
aadA24
aadA22
aadA17
aadA16
aadA15
aadA12
aadA11
aadA8b
aadA5
aadA3
aadA2
aadA1
aac(3)-IIa
aac(3)-IVa
aac(3)-IIe
aph(3')-IIIa
aac(6')-Ib

(C)

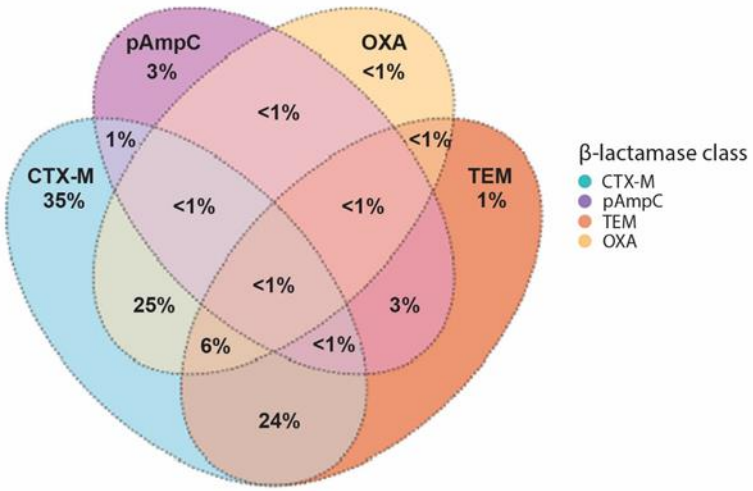

pAmpC
3%
OXA
<1%
CTX-M
35%
TEM
1%
<1%
1%
<1%
<1%
<1%
<1%
25%
3%
6%
<1%
24%
β-lactamase class
CTX-M
pAmpC
TEM
OXA

Figure 2: Replicon types, β-lactamase genes, and other horizontally acquired resistance determinants in ESCR UPEC (N=577), identified from the WGS analysis. (A) Chord diagram displaying the replicon types and their association with β-lactamase classes. Chord plot displays replicon types/β-lactamase genes as a proportion of all replicon-gene combinations. (B) Sunburst plot displaying proportions (presented on a logarithmic scale) of horizontally acquired resistance mechanisms which provide protection against agents other than β-lactams. The bar representing the gene *aac(6')-Ib-cr* is labeled both peach and lighbt green, as this determinant confers resistance to both fluoroquinolones and aminoglycosides. (C) Venn diagram displaying relationships between prevalent β-lactamase gene classes and the percentage of isolates in which each gene class was identified. Diagrams were created with the chordDiagram, ggplot2, and VennDiagram packages using R 3.0.1.

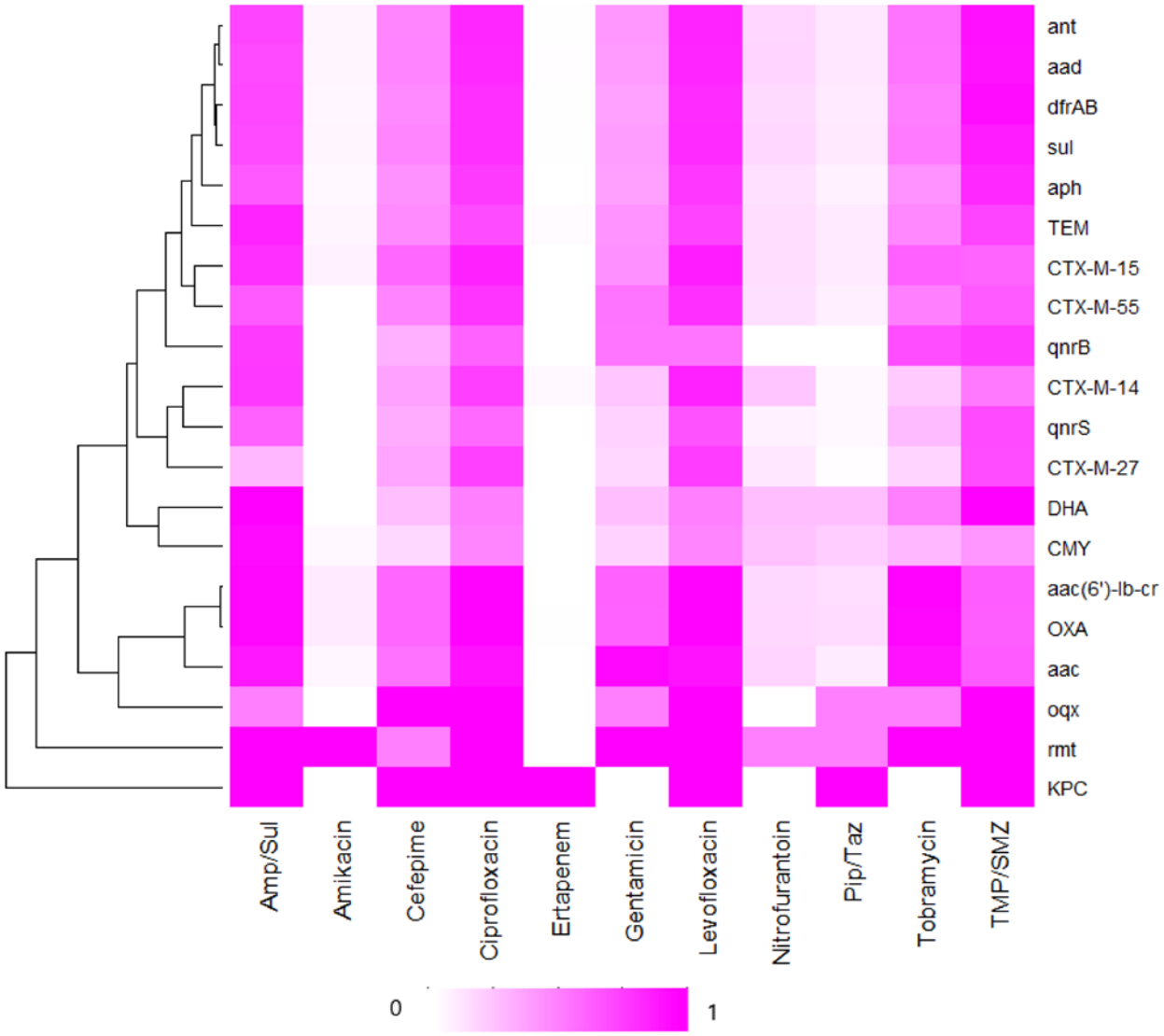


Figure 4: Heatmap displaying positive predictive value (PPV) of the presence of antibiotic resistance genes and phenotypic non-susceptibility to antimicrobial agents used in the treatment of UTI. The PPV of each genotype-phenotype classification was calculated and visually displayed in the form of a heatmap. A PPV of 0 is shown in white, whereas a PPV of 1 is dark pink. Ninety-five percent confidence intervals were calculated by bootstrapping. Hierarchical clustering was applied to the genes included in this analysis. The heatmap was created with the heatmap package using R 3.0.1.

Antimicrobial agent	Risk Ratio	95% Confidence Interval	*p*
Ampicillin-sulbactam	1.71	(1.56 - 1.88)	**<0.001**
Amikacin	8.08	(2.72 - 23.98)	**<0.001**
Cefepime	1.34	(1.14 - 1.56)	**0.001**
Ciprofloxacin	1.33	(1.25 - 1.42)	**<0.001**
Levofloxacin	1.26	(1.19 - 1.34)	**<0.001**
Gentamicin	2.46	(2.00 - 3.04)	**<0.001**
Nitrofurantoin	1.33	(0.85 - 2.10)	0.212
Piperacillin-tazobactam	3.41	(1.70 - 6.87)	**0.001**
Tobramycin	3.76	(3.16 - 4.48)	**<0.001**
Trimethoprim-sulfamethoxazole	0.99	(0.85 - 1.14)	0.923
MDR	1.79	(1.63 - 1.98)	**<0.001**

Table 3. Risk of non-susceptibility (intermediate or resistant phenotype) associated with the co-occurrence of *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{OXA-1}}$, and *aac(6')-Ib-cr* within isolates with an ESBL phenotype. Risk Ratios were calculated using unconditional maximum likelihood estimation, and 95%. Confidence intervals were calculated using normal approximation.

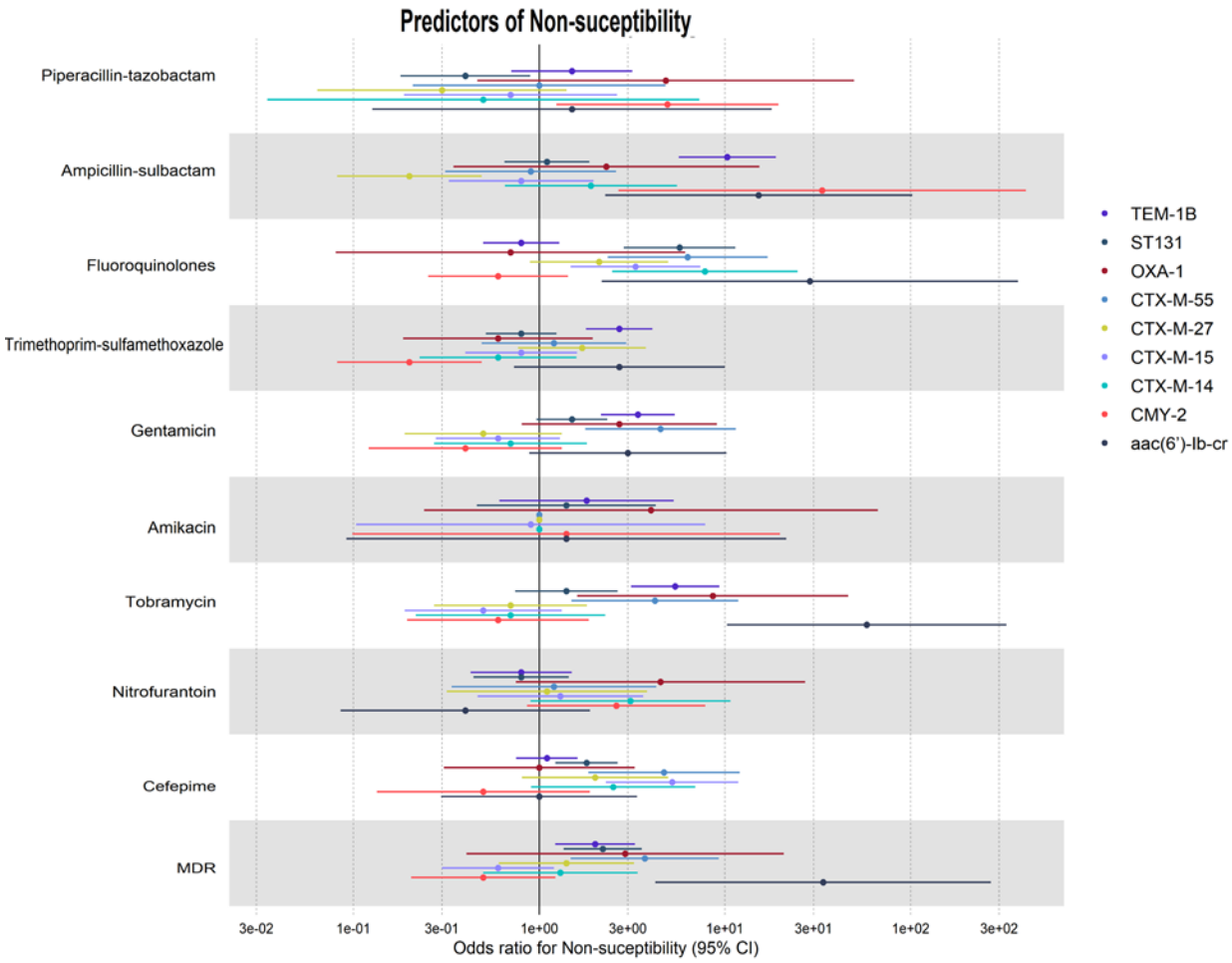


Figure 5: Forest plot displaying results of logistic regression analysis to assess the presence of resistance genes of interest and the ST131 lineage, as predictors of antibiotic non-susceptibility and MDR in UTI. Generalized linear model used a logit link function. Outcomes of the 10 models are binary (1 or 0), with 1 denoting non-susceptibility or MDR status as defined as non-susceptible to ≥ 3 classes of antimicrobials included in this analysis. Covariates in each model include; ST131, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CMY-2}}$ and *aac(6')-lb-cr*.

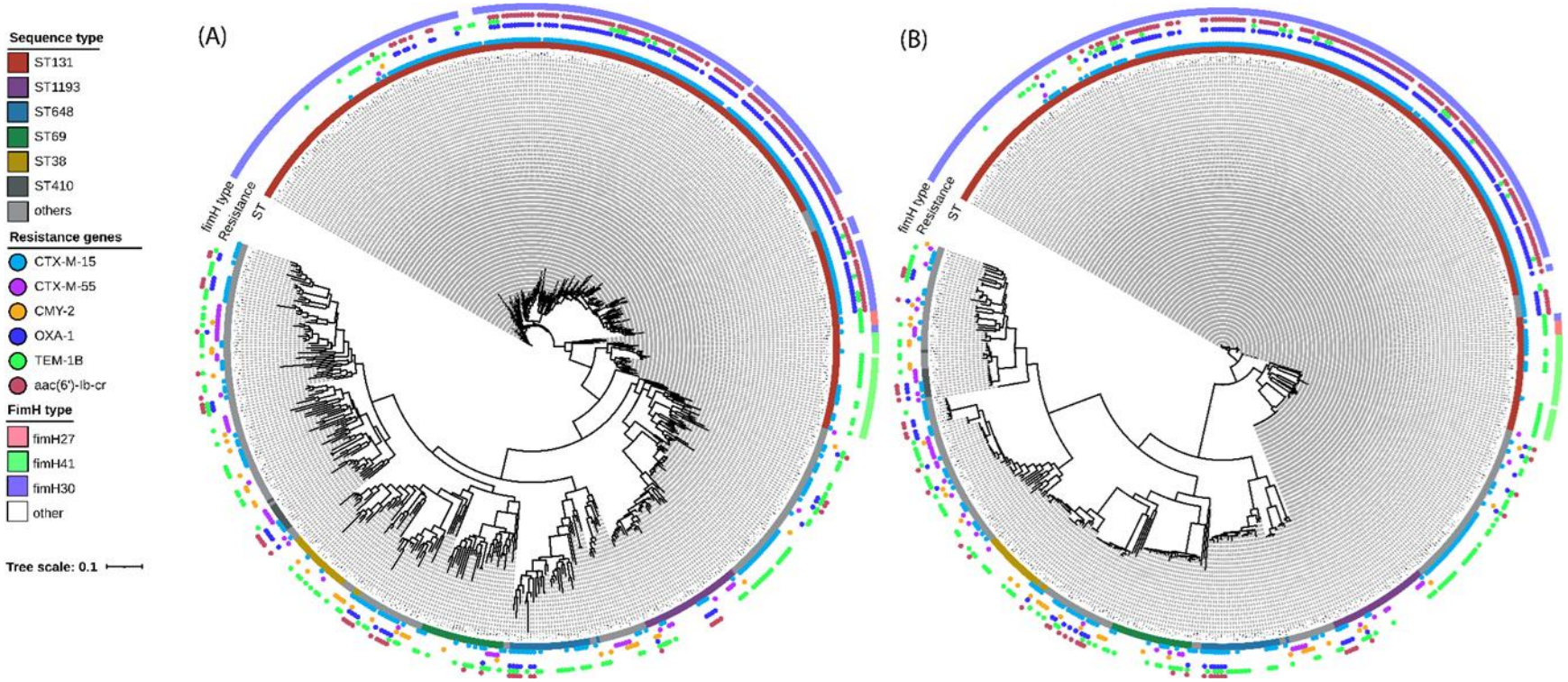


Figure 6: Maximum likelihood phylogenetic trees of the ESCR UPEC in this collection, displaying *fimH* type, MLST, and β-lactamase/resistance genes of interest. Trees display phylogenetic analysis based on the (A) accessory genome and (B) SNP information from pangenome analyses. Isolate sequence type is highlighted by the color-coded internal bar (‘ ST’), the presence of resistance genes of interest is denoted by the colored circles (‘ Resistance’), and the *fimH* type is displayed by the external-colored bar framing the phylogenetic tree (‘ fimH type’).

Supplementary information

Supplementary Figure S1: (A) Proportions of phylogroups identified, and (B) pie plot displaying prevalence of MLSTs, sorted by clinical laboratory site (Sites 1-6). Diagrams were created using ggplot2 in R 3.0.1.

Supplementary Table S1: Antimicrobial non-susceptibility (intermediate or resistant MIC values), stratified by phenotypic ESBL status. Statistical analyses were performed using Fisher's exact test in R 3.0.1.

Supplementary Table S2: Antimicrobial non-susceptibility (intermediate or resistant MIC values), stratified by the 6 most common MLST types identified in this collection.

Supplementary Table S3: Antimicrobial resistance profiles, stratified by phenotypic ESBL status. Statistical analyses were performed using Fisher's exact test in R 3.0.1. Abbreviations are defined as the following, BL = β-lactam, N = nitrofurantoin, FQ = fluoroquinolones, AG = aminoglycosides, TS = trimethoprim-sulfamethoxazole.

Supplementary Table S4: β-lactamase genes identified from WGS analysis, stratified by ESBL phenotype. Statistical analyses were performed using the Fisher's exact test in R 3.0.1.

Supplementary Table S5: Horizontally acquired resistance genes and mutations conferring antibiotic resistance, identified from WGS analysis, and stratified by ESBL phenotype. Statistical analyses were performed using the Fisher's exact test in R 3.0.1.

Supplementary Table S6: Plasmid replicon types identified from WGS analysis, stratified by ESBL phenotype. Statistical analyses were performed using Fischer's exact test in R 3.0.1.

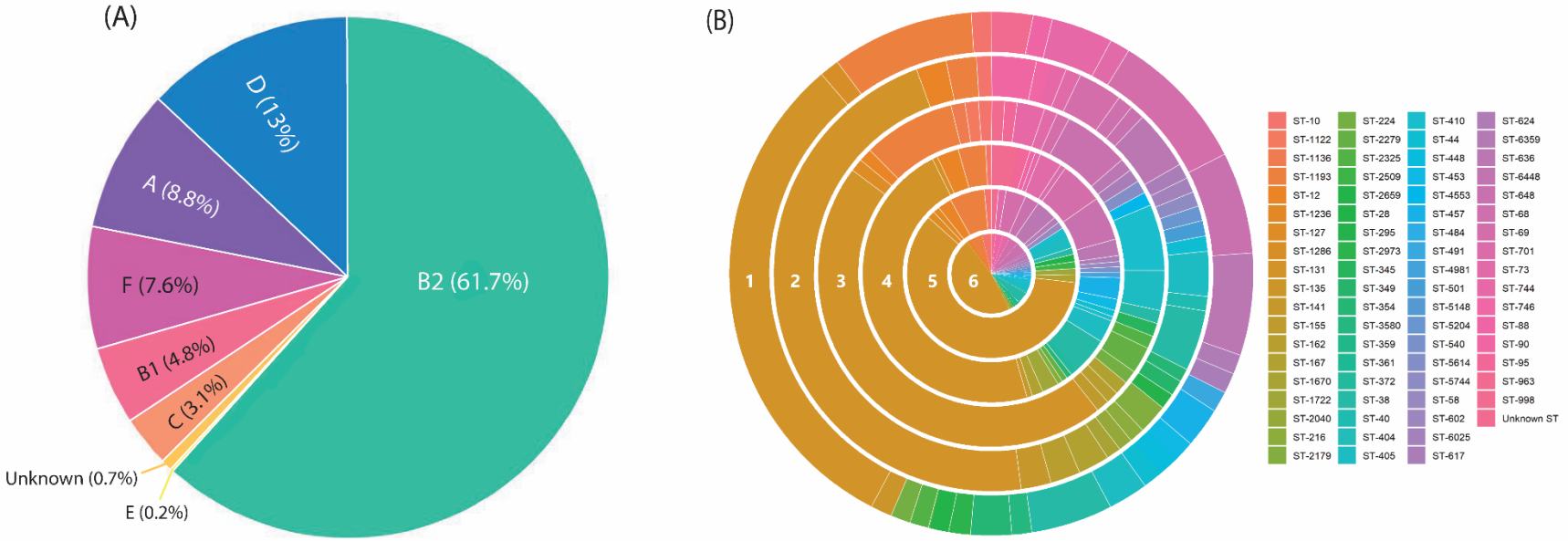
Supplementary Figure S2: Correlation between β-lactamase genes and horizontally acquired resistance determinants, detected in ESCR UPEC from WGS analysis. (A) Resistance gene data were analyzed using the Phi correlation coefficient, for all ESCR UPEC (N=577). Genes ecoding proteins with the same mechanism of action were grouped according to their class. (B) To examine relationships between common variants of *bla*$_{\text{CTX-M}}$ and other horizontally acquired resistance genes, we analyzed the 527 isolates with ESBL phenotypes. Phi correlation coefficients can be interpreted as: 0 = no relationship, ± <0.29 = weak, between ± 0.3 and ± 0.49 = moderate, between ± 0.5 and ± 0.99 = strong, ± 1 = perfect. The ellipses surrounding correlation coefficients display the confidence regions of the distribution and strength of correlation. The figure was generated using the corrplot and ggplot2 functions in R 3.0.1.

Supplementary Table S7: Matrix displaying positive predictive values (PPVs) for resistance genes of interest vs. antimicrobial non-susceptibility. Isolates which displayed intermediate or resistant MIC values in relation to CLSI breakpoints were grouped and described as 'non-susceptible' for this analysis. Abbreviated drug names correspond to: Amp-Sul = ampicillin-sulbactam, Pip-Taz = piperacillin-tazobactam, TMP-SMZ = trimethoprim-sulfamethoxazole.

Supplementary Table S8: Logistic regression analysis to assess the presence of common resistance genes as predictors of antibiotic non-susceptibility and MDR in ESCR UPEC. Generalized linear model used a logit link function and the glm(family = binomial) function in R. Outcomes are binary (1 or 0), with 1 denoting non susceptibility or MDR status (defined as resistant to at least 1 agent in ≥3 classes of antimicrobial agents). The most common β-lactamase

genes, the MLST type, ST-131, and the acetyltransferase gene, *aac(6')-Ib-cr*, were included in the analysis. Abbreviated drug names correspond to: Pip-Taz = piperacillin-tazobactam, FQ = fluoroquinolones, TMP-SMZ = trimethoprim-sulfamethoxazole, NIT = nitrofurantoin.

Supplementary Table S9: Logistic regression analysis to assess the presence of common resistance genes as predictors of antibiotic non-susceptibility to fluoroquinolones in ESCR UPEC. Generalized linear model used a logit link function and the glm(family = binomial) function in R. Outcomes are binary (1 or 0).



Supplementary Figure S1: (A) Proportions of phylogroups identified, and (B) pie plot displaying prevalence of MLSTs, sorted by clinical laboratory site (Sites 1-6). Diagrams were created using ggplot2 in R 3.0.1

Supplementary Table S1: Antimicrobial non-susceptibility (intermediate or resistant MIC values), stratified by phenotypic ESBL status. Statistical analyses were performed using Fisher's exact test in R 3.0.1.

Antimicrobial non-susceptibility	**Non-ESBL (N=50)**	**ESBL (N=527)**	**Overall (N=577)**	***p***
Trimethoprim-sulfamethoxazole	22 (44.0%)	328 (62.2%)	350 (60.7%)	0.239
Nitrofurantoin	10 (20.0%)	68 (12.9%)	78 (13.5%)	1
Levofloxacin	20 (40.0%)	444 (84.3%)	464 (80.4%)	**<0.001**
Ciprofloxacin	20 (40.0%)	431 (81.8%)	451 (78.2%)	**<0.001**
Tobramycin	11 (22.0%)	258 (49.0%)	269 (46.6%)	**0.004**
Gentamicin	11 (22.0%)	197 (37.4%)	208 (36.0%)	0.501
Amikacin	0 (0%)	19 (3.6%)	19 (3.3%)	1
Ertapenem	1 (2.0%)	2 (0.4%)	3 (0.5%)	-
Piperacillin-tazobactam	9 (18.0%)	31 (5.9%)	40 (6.9%)	0.072
Ampicillin-sulbactam	44 (88.0%)	366 (69.4%)	410 (71.1%)	0.081
Cefepime	4 (8.0%)	269 (51.0%)	273 (47.3%)	**<0.001**
Cefotaxime	39 (78.0%)	525 (99.6%)	564 (97.7%)	**<0.001**
Ceftriaxone	49 (98.0%)	525 (99.6%)	574 (99.5%)	1
Ceftazidime	42 (84.0%)	377 (71.5%)	419 (72.6%)	1
MDR	20 (40.0%)	368 (69.8%)	388 (67.2%)	**<0.001**

Supplementary Table S2: Antimicrobial non-susceptibility (intermediate or resistant MIC values), stratified by the 6 most common MLST types identified in this collection.

Antimicrobial non-susceptibility	MLST					
	ST131 (N=267)	ST1193 (N=32)	ST38 (N=22)	ST636 (N=18)	ST648 (N=26)	ST69 (N=26)
Trimethoprim-sulfamethoxazole	161 (60.3%)	22 (68.8%)	12 (54.5%)	14 (77.8%)	16 (61.5%)	17 (65.4%)
Nitrofurantoin	32 (12.0%)	3 (9.4%)	4 (18.2%)	0 (0%)	10 (38.5%)	0 (0%)
Levofloxacin	252 (94.4%)	32 (100%)	11 (50.0%)	8 (44.4%)	26 (100%)	12 (46.2%)
Ciprofloxacin	253 (94.8%)	32 (100%)	10 (45.5%)	5 (27.8%)	26 (100%)	10 (38.5%)
Tobramycin	160 (59.9%)	10 (31.3%)	0 (0%)	2 (11.1%)	14 (53.8%)	7 (26.9%)
Gentamicin	116 (43.4%)	9 (28.1%)	2 (9.1%)	0 (0%)	9 (34.6%)	6 (23.1%)
Amikacin	13 (4.9%)	1 (3.1%)	0 (0%)	0 (0%)	1 (3.8%)	0 (0%)
Ertapenem	2 (0.7%)	0 (0%)	0 (0%)	0 (0%)	1 (3.8%)	0 (0%)
Piperacillin-tazobactam	17 (6.4%)	0 (0%)	0 (0%)	0 (0%)	3 (11.5%)	1 (3.8%)
Ampicillin-sulbactam	195 (73.0%)	18 (56.3%)	12 (54.5%)	11 (61.1%)	23 (88.5%)	13 (50.0%)
Cefepime	13 (40.6%)	154 (57.7%)	9 (40.9%)	6 (33.3%)	14 (53.8%)	7 (26.9%)
Cefotaxime	260 (97.4%)	31 (96.9%)	22 (100%)	18 (100%)	26 (100%)	24 (92.3%)
Ceftriaxone	266 (99.6%)	32 (100%)	22 (100%)	18 (100%)	26 (100%)	26 (100%)
Ceftazidime	184 (68.9%)	25 (78.1%)	15 (68.2%)	13 (72.2%)	17 (65.4%)	19 (73.1%)
MDR	218 (81.6%)	25 (78.1%)	8 (36.4%)	0 (0%)	19 (73.1%)	9 (34.6%)
ESBL phenotype	260 (97.4%)	29 (90.6%)	20 (90.9%)	18 (100%)	25 (96.2%)	22 (84.6%)

Supplementary Table S3: Antimicrobial resistance profiles, stratified by phenotypic ESBL status. Statistical analyses were performed using Fisher's exact test in R 3.0.1. Abbreviations are defined as the following, BL = β-lactam, N = nitrofurantoin, FQ = fluoroquinolones, AG = aminoglycosides, TS = trimethoprim-sulfamethoxazole.

Resistance classes	**Non-ESBL (N=50)**	**ESBL (N=527)**	**Overall (N=577)**	***p***
BL	18 (36%)	43 (8.1%)	61 (10.6%)	**<0.001**
BL, N	3 (6.0%)	3 (0.6%)	6 (1.0%)	0.063
BL, FQ	2 (4%)	70 (13.3%)	72 (12.5%)	0.991
BL, TS	6 (12%)	42 (8%)	48 (8.3%)	1
BL, AG	1 (2%)	1 (0.2%)	2 (0.3%)	0.5
BL, FQ, N	0 (0%)	2 (0.4%)	2 (0.3%)	-
BL, FQ, AG	4 (8%)	76 (14.4%)	80 (13.9%)	1
BL, FQ, TS	5 (10%)	102 (19.4%)	107 (18.6%)	1
BL, AG, TS	1 (2%)	21 (4%)	22 (3.9%)	1
BL, AG, N, TS	1 (2%)	0 (0%)	1 (0.2%)	-
BL, FQ, N, TS	2 (4%)	8 (1.5%)	10 (1.7%)	1
BL, FQ, AG, TS	6 (12%)	144 (27.3%)	150 (26%)	1
BL, FQ, AG, N	0 (0%)	4 (0.8%)	4 (0.7%)	-
BL, FQ, AG, N, TS	1 (2%)	11 (2.1%)	12 (2%)	1

Supplementary Table S4: β-lactamase genes identified from WGS analysis, stratified by ESBL phenotype. Statistical analyses were performed using the Fisher's exact test in R 3.0.1.

β-lactamase classification	Family	Activity	Gene	non-ESBL (N=50)	ESBL (N=527)	Total (N=577)	*p*
Class A	**CTX-M**	ESBL	**All CTX-M**	6 (12.0%)	524 (99.4%)	530 (91.8%)	**<0.001**
		ESBL	CTX-M-1	0 (0%)	3 (0.6%)	3 (0.5%)	-
		ESBL	CTX-M-3	0 (0%)	5 (0.9%)	5 (0.9%)	1
		ESBL	CTX-M-14	0 (0%)	44 (8.3%)	44 (7.6%)	1
		ESBL	CTX-M-15	3 (6.0%)	321 (60.9%)	324 (56.2%)	**<0.001**
		ESBL	CTX-M-24	0 (0%)	1 (0.2%)	1 (0.2%)	-
		ESBL	CTX-M-27	2 (4.0%)	94 (17.8%)	96 (16.6%)	0.686
		ESBL	CTX-M-32	0 (0%)	1 (0.2%)	1 (0.2%)	-
		ESBL	CTX-M-55	0 (0%)	48 (9.1%)	48 (8.3%)	1
		ESBL	CTX-M-64	0 (0%)	1 (0.2%)	1 (0.2%)	-
		ESBL	CTX-M-65	0 (0%)	6 (1.1%)	6 (1.0%)	1
		ESBL	CTX-M-201	1 (2.0%)	0 (0%)	1 (0.2%)	-
	TEM	ESBL	TEM-10	0 (0%)	1 (0.2%)	1 (0.2%)	-
		ESBL	TEM-15	1 (2.0%)	0 (0%)	1 (0.2%)	-
		Narrow-spectrum	**All narrow-spectrum TEM**	20 (40%)	187 (35.48%)	207 (44.9%)	1
		Narrow-spectrum	TEM-1A	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	TEM-1B	17 (34.0%)	152 (28.8%)	169 (29.3%)	1
		Narrow-spectrum	TEM-1C	1 (2.0%)	3 (0.6%)	4 (0.7%)	-
		Narrow-spectrum	TEM-35	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	TEM-76	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	TEM-104	0 (0%)	2 (0.4%)	2 (0.3%)	-
		Narrow-spectrum	TEM-176	1 (2.0%)	0 (0%)	1 (0.2%)	-
		Narrow-spectrum	TEM-209	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	TEM-214	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	TEM-216	0 (0%)	4 (0.8%)	4 (0.7%)	-
		Narrow-spectrum	TEM-220	1 (2.0%)	0 (0%)	1 (0.2%)	-
		Narrow-spectrum	TEM-232	0 (0%)	15 (2.8%)	15 (2.6%)	1
		Narrow-spectrum	TEM-234	0 (0%)	6 (1.1%)	6 (1.0%)	1
	SHV	ESBL	SHV-12	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	SHV-198	1 (2.0%)	1 (0.2%)	2 (0.3%)	-

	CARB	Carbenicillinase	CARB-2	1 (2.0%)	1 (0.2%)	2 (0.3%)	-
	KPC	Carbapenemase	KPC-2	0 (0%)	1 (0.2%)	1 (0.2%)	-
Class C	**CMY**	pAmpC cephalosporinase	**All CMY**	33 (66%)	15 (2.85%)	48 (8.31%)	**<0.001**
		pAmpC cephalosporinase	CMY-2	30 (60.0%)	10 (1.9%)	40 (6.9%)	**<0.001**
		pAmpC cephalosporinase	CMY-4	1 (2.0%)	0 (0%)	1 (0.2%)	-
		pAmpC cephalosporinase	CMY-42	1 (2.0%)	0 (0%)	1 (0.2%)	-
		pAmpC cephalosporinase	CMY-130	1 (2.0%)	5 (0.9%)	6 (1.0%)	1
	DHA	pAmpC cephalosporinase	DHA-1	1 (2.0%)	2 (0.4%)	3 (0.5%)	-
		pAmpC cephalosporinase	DHA-6	1 (2.0%)	0 (0%)	1 (0.2%)	-
Class D	**OXA**	Narrow-spectrum	**All OXA**	4 (8.0%)	181 (34.35%)	185 (32.1%)	**0.004**
		Narrow-spectrum	OXA-1	4 (8.0%)	177 (33.6%)	181 (31.4%)	**0.007**
		Narrow-spectrum	OXA-9	0 (0%)	1 (0.2%)	1 (0.2%)	-
		Narrow-spectrum	OXA-10	0 (0%)	2 (0.4%)	2 (0.3%)	-
		Narrow-spectrum	OXA-320	0 (0%)	1 (0.2%)	1 (0.2%)	-

Supplementary Table S5: Horizontally acquired resistance genes and mutations conferring antibiotic resistance, identified from WGS analysis, and stratified by ESBL phenotype. Statistical analyses were performed using the Fisher's exact test in R 3.0.1.

Resistance conferred (*gene groupings for correlation analysis*)	**Gene**	**non-ESBL (N=50)**	**ESBL (N=527)**	**Total (N=577)**	***p***
Sulfonamides *(sul)*	*sul1*	16 (32.0%)	270 (51.2%)	286 (49.6%)	0.981
	sul2	20 (40.0%)	216 (41.0%)	236 (40.9%)	1
	sul3	2 (4.0%)	30 (5.7%)	32 (5.5%)	1
Trimethoprim *(dfrA/B)*	*drfA1*	3 (6.0%)	27 (5.1%)	30 (5.2%)	1
	drfA5	1 (2.0%)	9 (1.7%)	10 (1.7%)	1
	drfA8	1 (2.0%)	2 (0.4%)	3 (0.5%)	-
	drfA12	7 (14.0%)	33 (6.3%)	40 (6.9%)	1
	dfrA14	3 (6.0%)	39 (7.4%)	42 (7.3%)	1
	drfA16	0 (0%)	1 (0.2%)	1 (0.2%)	-
	drfA17	11 (22.0%)	236 (44.8%)	247 (42.8%)	0.239
	drfA19	0 (0%)	2 (0.4%)	2 (0.3%)	-
	drfA27	0 (0%)	3 (0.6%)	3 (0.5%)	-
	drfA32	0 (0%)	1 (0.2%)	1 (0.2%)	-
	drfB4	0 (0%)	1 (0.2%)	1 (0.2%)	-
MDR efflux pump *(oqx)*	*oqxAB*	0 (0%)	2 (0.4%)	2 (0.3%)	-
Fluoroquinolones *(qnrB, qnrS)*	*qnrB1*	0 (0%)	4 (0.8%)	4 (0.7%)	-
	qnrB2	0 (0%)	1 (0.2%)	1 (0.2%)	-
	qnrB4	1 (2.0%)	2 (0.4%)	3 (0.5%)	-
	qnrB19	0 (0%)	5 (0.9%)	5 (0.9%)	1
	qnrS1	2 (4.0%)	29 (5.5%)	31 (5.4%)	1
	qnrS2	0 (0%)	3 (0.6%)	3 (0.5%)	-
	mdfA	50 (100%)	527 (100%)	577 (100%)	-
	gyrA mutation D87G	0 (0%)	2 (0.4%)	2 (0.3%)	-
	gyrA mutation D87N	16 (32.0%)	361 (68.5%)	377 (65.3%)	**<0.001**
	gyrA mutation D87Y	1 (2.0%)	9 (1.7%)	10 (1.7%)	1
	gyrA mutation S83A	0 (0%)	1 (0.2%)	1 (0.2%)	-
	gyrA mutation S83L	18 (36.0%)	438 (83.1%)	456 (79.0%)	**<0.001**
	parC mutation A56T	0 (0%)	9 (1.7%)	9 (1.6%)	1
	parC mutation E84G	1 (2.0%)	11 (2.1%)	12 (2.1%)	1
	parC mutation E84V	5 (10.0%)	229 (43.5%)	234 (40.6%)	**<0.001**
	parC mutation S57T	0 (0%)	3 (0.6%)	3 (0.5%)	-
	parC mutation S80I	17 (34.0%)	382 (72.5%)	399 (69.2%)	**<0.001**
	parC mutation S80R	0 (0%)	4 (0.8%)	4 (0.7%)	-
	parE mutation E460D	0 (0%)	2 (0.4%)	2 (0.3%)	-
	parE mutation I355T	2 (4.0%)	3 (0.6%)	5 (0.9%)	1
	parE mutation I529L	6 (12.0%)	247 (46.9%)	253 (43.8%)	**<0.001**

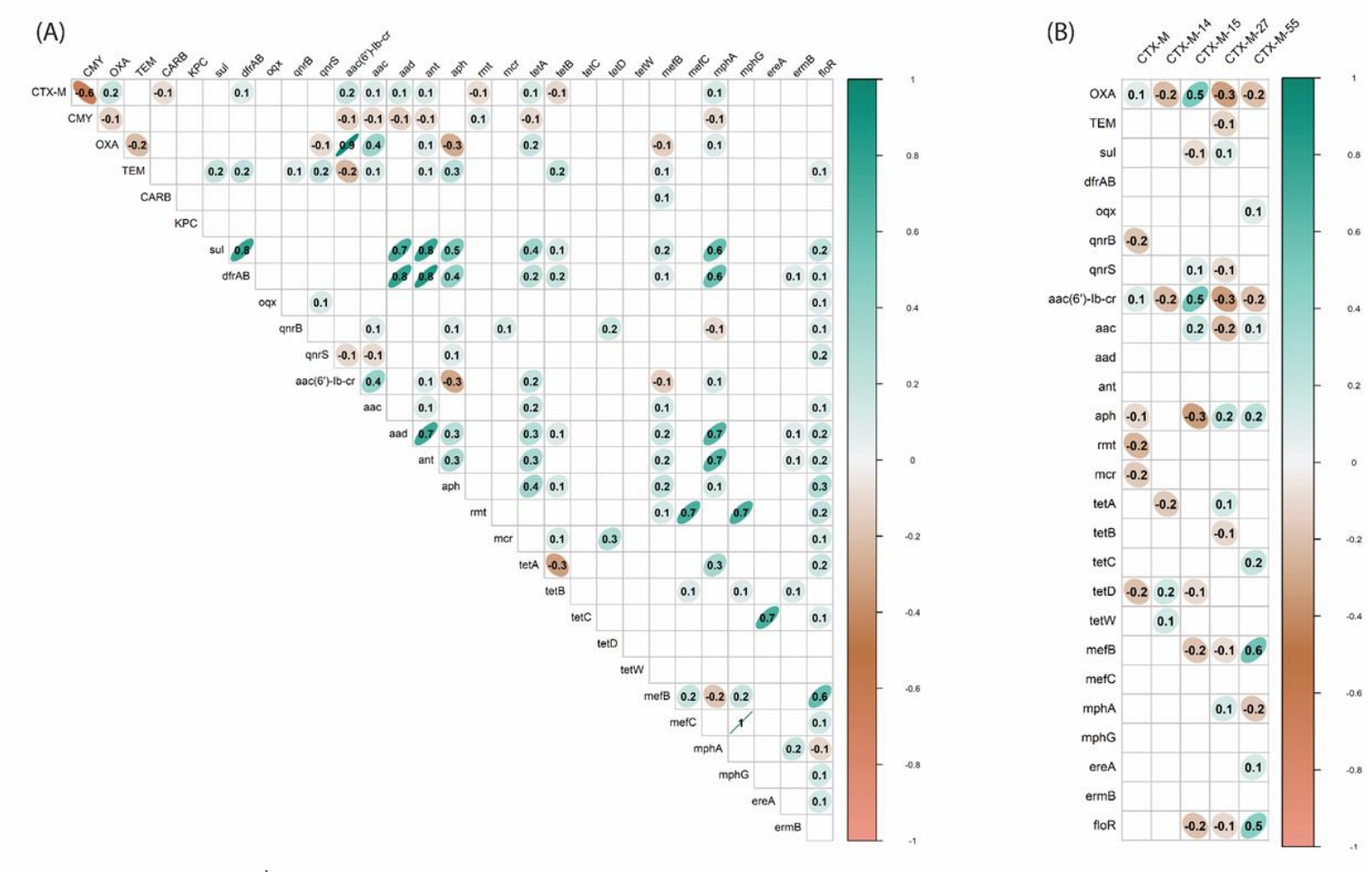
	parE mutation L416F	5 (10.0%)	33 (6.3%)	38 (6.6%)	1
	parE mutation L445H	1 (2.0%)	1 (0.2%)	2 (0.3%)	-
	parE mutation S458A	5 (10.0%)	72 (13.7%)	77 (13.3%)	1
	parE mutation S458T	0 (0%)	4 (0.8%)	4 (0.7%)	-
Fluoroquinolone/ aminoglycoside	*aac(6')-Ib-cr*	4 (8.0%)	182 (34.5%)	186 (32.2%)	**0.004**
Aminoglycosides *(aac, aad, ant, aph, rmt)*	*aac(3)-IIa*	2 (4.0%)	137 (26.0%)	139 (24.1%)	**0.026**
	aac(3)-IId	7 (14.0%)	55 (10.4%)	62 (10.7%)	1
	aac(3)-IIe	1 (2.0%)	2 (0.4%)	3 (0.5%)	-
	aac(3)-IVa	0 (0%)	2 (0.4%)	2 (0.3%)	-
	aac(3)-VIa	0 (0%)	1 (0.2%)	1 (0.2%)	-
	aadA1	1 (2.0%)	28 (5.3%)	29 (5.0%)	1
	aadA2	8 (16.0%)	39 (7.4%)	47 (8.1%)	1
	aadA3	2 (4.0%)	20 (3.8%)	22 (3.8%)	1
	aadA5	8 (16.0%)	229 (43.5%)	237 (41.1%)	0.076
	aadA8b	2 (4.0%)	10 (1.9%)	12 (2.1%)	1
	aadA11	0 (0%)	1 (0.2%)	1 (0.2%)	-
	aadA12	1 (2.0%)	1 (0.2%)	2 (0.3%)	1
	aadA15	1 (2.0%)	22 (4.2%)	23 (4.0%)	1
	aadA16	0 (0%)	3 (0.6%)	3 (0.5%)	-
	aadA17	0 (0%)	3 (0.6%)	3 (0.5%)	-
	aadA22	0 (0%)	8 (1.5%)	8 (1.4%)	1
	aadA24	2 (4.0%)	17 (3.2%)	19 (3.3%)	1
	ant(2'')-Ia	1 (2.0%)	8 (1.5%)	9 (1.6%)	1
	ant(3'')-Ia	24 (48.0%)	330 (62.6%)	354 (61.4%)	1
	ant(3'')-Ii/aac(6')-IId	0 (0%)	2 (0.4%)	2 (0.3%)	-
	aph(3'')-Ib	22 (44.0%)	205 (38.9%)	227 (39.3%)	1
	aph(3')-Ia	6 (12.0%)	50 (9.5%)	56 (9.7%)	1
	aph(3')-IIa	0 (0%)	1 (0.2%)	1 (0.2%)	-
	aph(4)-a	0 (0%)	2 (0.4%)	2 (0.3%)	-
	aph(6)-Id	22 (44.0%)	210 (39.8%)	232 (40.2%)	1
	rmtB	0 (0%)	1 (0.2%)	1 (0.2%)	-
	rmtE	0 (0%)	1 (0.2%)	1 (0.2%)	-
Polymyxins	*mcr*	0 (0%)	4 (0.8%)	4 (0.7%)	-
	pmrA mutation R81S	0 (0%)	1 (0.2%)	1 (0.2%)	-
Tetracyclines	*tetA*	15 (30.0%)	276 (52.4%)	291 (50.4%)	0.324
	tetB	15 (30.0%)	85 (16.1%)	100 (17.3%)	1
	tetC	0 (0%)	2 (0.4%)	2 (0.3%)	-
	tetD	0 (0%)	3 (0.6%)	3 (0.5%)	-
	tetW	0 (0%)	1 (0.2%)	1 (0.2%)	-
	16s rRNA *rrsB* mutation G1058C	0 (0%)	1 (0.2%)	1 (0.2%)	-
Macrolides	*mefB*	2 (4.0%)	27 (5.1%)	29 (5.0%)	1
	mefC	0 (0%)	1 (0.2%)	1 (0.2%)	-

	mphA	13 (26.0%)	263 (49.9%)	276 (47.8%)	0.51
	mphG	0 (0%)	1 (0.2%)	1 (0.2%)	-
	ereA	0 (0%)	1 (0.2%)	1 (0.2%)	-
	ermB	1 (2.0%)	29 (5.5%)	30 (5.2%)	1
Florfenicol	*floR*	3 (6.0%)	40 (7.6%)	43 (7.5%)	1
Cephalosporins	Chromosomal *ampC* promoter mutation, T-32A	1 (2.0%)	0 (0%)	1 (0.2%)	-
	Chromosomal ampC promoter mutation, C-42T	4 (8.0%)	0 (0%)	4 (0.7%)	-

Supplementary Table S6: Plasmid replicon types identified from WGS analysis, stratified by ESBL phenotype. Statistical analyses were performed using Fischer's exact test in R 3.0.1.

Replicon type	Non-ESBL (N=50)	ESBL (N=527)	Overall (N=577)	*p*
Col	27 (54.0%)	362 (68.7%)	389 (67.4%)	0.5
IncAC	2 (4.0%)	1 (0.2%)	3 (0.5%)	-
IncBO	3 (6.0%)	35 (6.6%)	38 (6.6%)	1
IncFIA	19 (38.0%)	323 (61.3%)	342 (59.3%)	**0.041**
IncFIB	34 (68.0%)	401 (76.1%)	435 (75.4%)	1
IncFIC	12 (24.0%)	157 (29.8%)	169 (29.3%)	1
IncFII	28 (56.0%)	382 (72.5%)	410 (71.1%)	0.383
IncHI	17 (34.0%)	57 (10.8%)	74 (12.8%)	**<0.001**
IncI	3 (6.0%)	14 (2.7%)	17 (2.9%)	1
IncLM	0 (0%)	1 (0.2%)	1 (0.2%)	-
IncN	3 (6.0%)	18 (3.4%)	21 (3.6%)	1
IncP	0 (0%)	1 (0.2%)	1 (0.2%)	-
IncQ	8 (16.0%)	40 (7.6%)	48 (8.3%)	1
IncR	1 (2.0%)	3 (0.6%)	4 (0.7%)	-
IncX	8 (16.0%)	39 (7.4%)	47 (8.1%)	0.933
IncY	4 (8.0%)	31 (5.9%)	35 (6.1%)	1

Supplementary Figure S2: Correlation between β-lactamase genes and horizontally acquired resistance determinants, detected in 3GCR UPEC from WGS analysis. (A) Resistance gene data were analyzed using the Phi correlation coefficient, for all 3GCR UPEC (N=577). Genes ecoding proteins with the same mechanism of action were grouped according to their class. (B) To examine relationships between common variants of *bla*$_{\text{CTX-M}}$ and other horizontally acquired resistance genes, we analyzed the 527 isolates with ESBL phenotypes. Phi correlation coefficients can be interpreted as: 0 = no relationship, ± <0.29 = weak, between ± 0.3 and ± 0.49 = moderate, between ± 0.5 and ± 0.99 = strong, ± 1 = perfect. The ellipses surrounding correlation coefficients display the confidence regions of the distribution and strength of correlation. The figure was generated using the corrplot and ggplot2 functions in R 3.0.1



Supplementary Table S7: Matrix displaying positive predictive values (PPVs) for resistance genes of interest vs. antimicrobial non-susceptibility. Isolates which displayed intermediate or resistant MIC values in relation to CLSI breakpoints were grouped and described as 'non-susceptible' for this analysis. Abbreviated drug names correspond to: Amp-Sul = ampicillin-sulbactam, Pip-Taz = piperacillin-tazobactam, TMP-SMZ = trimethoprim-sulfamethoxazole.

	Antimicrobial agent										
Gene	Amp-Sul	Amika-cin	Cefe-pime	Cipro-floxacin	Erta-penem	Genta-micin	Levo-floxacin	Nitro-furantoin	Pip-Taz	Tobra-mycin	TMP-SMZ
CTX-M-14	0.773	0.000	0.364	0.750	0.023	0.227	0.864	0.227	0.023	0.205	0.523
CTX-M-15	0.812	0.052	0.593	0.861	0.003	0.435	0.880	0.133	0.080	0.620	0.608
CTX-M-27	0.281	0.000	0.354	0.740	0.000	0.156	0.760	0.094	0.010	0.167	0.698
CTX-M-55	0.646	0.000	0.479	0.792	0.000	0.542	0.813	0.125	0.063	0.500	0.646
CMY class	0.957	0.022	0.152	0.478	0.000	0.174	0.478	0.239	0.196	0.283	0.413
DHA class	1.000	0.000	0.250	0.500	0.000	0.250	0.500	0.250	0.250	0.500	1.000
OXA class	0.962	0.082	0.598	0.973	0.005	0.614	0.973	0.158	0.136	0.962	0.630
TEM class	0.855	0.040	0.455	0.700	0.015	0.420	0.735	0.130	0.080	0.470	0.730
KPC class	1.000	0.000	1.000	1.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000
sul	0.706	0.039	0.475	0.810	0.008	0.382	0.831	0.156	0.086	0.519	0.886
dfrA/B	0.720	0.036	0.459	0.810	0.008	0.368	0.827	0.146	0.080	0.505	0.945
qnrB	0.769	0.000	0.308	0.615	0.000	0.538	0.538	0.000	0.000	0.692	0.769
qnrS	0.618	0.000	0.324	0.588	0.000	0.176	0.676	0.059	0.029	0.265	0.706
aac(6')-Ib-cr	0.968	0.081	0.597	0.984	0.000	0.618	0.984	0.151	0.129	0.978	0.634
aac	0.901	0.034	0.552	0.916	0.005	0.970	0.916	0.167	0.079	0.916	0.640
aad	0.709	0.036	0.480	0.832	0.009	0.396	0.850	0.165	0.087	0.538	0.916
ant	0.729	0.037	0.475	0.845	0.008	0.407	0.856	0.158	0.090	0.545	0.924
aph	0.649	0.027	0.429	0.764	0.000	0.371	0.784	0.120	0.054	0.425	0.838

Supplementary Table S8: Logistic regression analysis to assess the presence of common resistance genes as predictors of antibiotic non-susceptibility and MDR in ESCR UPEC. Generalized linear model used a logit link function and the glm(family = binomial) function in R. Outcomes are binary (1 or 0), with 1 denoting non susceptibility or MDR status (defined as resistant to at least 1 agent in ≥3 classes of antimicrobial agents). The most common β-lactamase genes, the MLST type, ST-131, and the acetyltransferase gene, *aac(6')-Ib-cr*, were included in the analysis. Abbreviated drug names correspond to: Pip-Taz = piperacillin-tazobactam, FQ = fluoroquinolones, TMP-SMZ = trimethoprim-sulfamethoxazole, NIT = nitrofurantoin.

Predictor	Pip-Tazo		Amp-Sul		FQ		TMP-SMZ		Gentamicin		Amikacin		Tobramycin		NIT		Cefepime		MDR	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
ST131	0.4 (0.3 - 1.5)	0.28	1.1 (0.7 - 2.0)	0.63	5.7 (3.0 - 12.0)	<0.001	0.8 (0.5 - 1.2)	0.3	1.5 (1.0 - 2.4)	0.06	1.4 (0.5 - 4.6)	0.61	1.4 (0.7 - 2.5)	0.322	0.8 (0.4 - 1.3)	0.34	1.8 (1.2 - 2.6)	0.005	2.2 (1.4 - 3.7)	0
CTX-M-14	0.5 (0.02 - 4.24)	0.59	1.9 (0.7 - 5.9)	0.243	7.8 (2.6 - 26.0)	<0.001	0.6 (0.2 - 1.4)	0.228	0.7 (0.3 - 2.0)	0.534	0 (-)	1	0.7 (0.2 - 2.1)	0.479	3.1 (0.9 - 10.7)	0.07	2.5 (0.9 - 6.9)	0.073	1.3 (0.5 - 3.4)	0.55
CTX-M-15	0.7 (0.2 - 2.8)	0.56	0.8 (0.3 -1.8)	0.545	3.3 (1.5 - 7.5)	0.004	0.8 (0.4 - 1.6)	0.483	0.6 (0.3 - 1.4)	0.267	0.9 (0.14 - 10.6)	0.89	0.5 (0.2 - 1.4)	0.186	1.3 (0.5 - 3.9)	0.66	5.2 (2.4 - 12.4)	<0.001	0.6 (0.3 - 1.2)	0.15
CTX-M-27	0.3 (0.1 - 2.2)	0.29	0.2 (0.1 - 0.6)	0.002	2.1 (0.9 - 5.0)	0.096	1.7 (0.8 - 3.9)	0.176	0.5 (0.2 - 1.4)	0.191	0 (-)	0.99	0.7 (0.3 - 2.0)	0.538	1.1 (0.3 - 3.6)	0.93	2 (0.8 - 4.9)	0.142	1.4 (0.6 - 3.2)	0.41
CTX-M-55	1.0 (0.2 - 4.6)	0.96	0.9 (0.3 - 2.5)	0.802	6.3 (2.4 - 17.4)	<0.001	1.2 (0.5 - 3.0)	0.631	4.5 (1.8 - 11.6)	0.002	0 (-)	1	4.2 (1.5 - 11.9)	0.006	1.2 (0.3 - 3.8)	0.79	4.7 (1.9 - 12.4)	0.001	3.7 (1.5 - 9.4)	0
CMY-2	4.9 (1.3 - 20.4)	**0.02**	33.4 (4.0 - 626.1)	**0.001**	0.6 (0.3 - 1.7)	**0.355**	0.2 (0.1 - 0.6)	**0.002**	0.4 (0.1 - 1.1)	**0.072**	1.4 (0.1 - 20.1)	**0.83**	0.6 (0.2 - 1.9)	**0.413**	2.6 (0.9 - 8.2)	**0.09**	0.5 (0.1 - 1.4)	**0.195**	0.5 (0.2 - 1.2)	**0.11**
TEM-1B	1.49 (0.7 - 3.14)	0.3	10.3 (5.8 - 19.3)	<0.001	0.8 (0.5 - 1.28)	0.3	2.7 (1.8 -4.1)	<0.001	3.4 (2.2 - 5.5)	<0.001	1.8 (0.6 - 5.2)	0.27	5.4 (3.2 - 9.6)	<0.001	0.8 (0.4-1.4)	0.42	1.1 (0.7 - 1.5)	0.922	2 (1.3 - 3.5)	0
OXA-1	4.8 (0.5 - 53.4)	0.22	2.3 (0.3 - 13.3)	0.382	0.7 (0.1 - 7.6)	0.727	0.6 (0.2 - 2.1)	0.474	2.7 (0.8 - 9.0)	0.104	4.0 (0.3 - 83.1)	0.4	8.6 (1.5 - 43.2)	0.01	4.5 (0.8 - 28.9)	0.11	1 (0.3 - 3.2)	0.988	2.9 (0.4 - 20.5)	0.29
aac(6')-Ib-cr	1.5 (0.1 - 14.1)	0.77	15.2 (2.7 - 121.5)	0.005	28.7 (2.4 - 418.7)	0.01	2.7 (0.8 - 10.9)	0.127	3.0 (0.9 - 10.4)	0.08	1.4 (0.1- 23.4)	0.84	58.1 (12.1 - 387.2)	<0.001	0.4 (0.1 - 2.2)	0.28	1 (0.3 - 3.4)	0.979	33.8 (4.9 - 313)	0

Supplementary Table S9: Logistic regression analysis to assess the presence of common resistance genes as predictors of antibiotic non-susceptibility to fluoroquinolones in ESCR UPEC. Generalized linear model used a logit link function and the glm(family = binomial) function in R. Outcomes are binary (1 or 0).

Predictors	**Odds Ratios**	**95% Confidence Interval**	***p***
ST131	3.06	(1.49 – 6.61)	**0.003**
CTX-M-14	3.73	(0.98 – 15.67)	0.061
CTX-M-15	2.64	(0.98 – 7.19)	0.055
CTX-M-27	1.07	(0.37 – 3.10)	0.896
CTX-M-55	2.92	(0.93 – 9.88)	0.073
CMY-2	0.61	(0.19 – 2.00)	0.410
TEM-1B	0.36	(0.19 – 0.66)	**0.001**
OXA-1	0.23	(0.03 – 2.90)	0.204
aac(6')-Ib-cr	56.48	(4.08 – 865.12)	**0.003**
gyrase and/or topoisomerase IV mutations	16.42	(8.84 – 31.77)	**<0.001**

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CHAPTER 3. Spatial clusters of dominant lineages of uropathogenic *Escherichia coli* identified in a large San Francisco Healthcare system

INTRODUCTION

Community acquired urinary tract infections (CA-UTIs) are exceedingly common infections worldwide. An estimated 150 million people develop urinary tract infections globally every year. These infections are associated with significant clinical and economic burdens to patients and healthcare systems [1]. Antimicrobial resistance (AMR) is a critical challenge in the clinical management of UTIs. In 2019, UTIs were found to be the 4th leading cause of death associated with bacterial AMR [2]. While an increase in multidrug resistant (MDR) UTIs has long been recognized in hospital settings, evidence of an increase in MDR UTIs arising in community settings is unprecedented and highly concerning [2,3].

Although the most common cause of CA-UTIs is *E. coli*, as a taxonomic group, *E. coli* itself represents a broad category of organisms that vary significantly. Some strains of *E. coli* are considered healthy human commensal flora, while others are associated with UTIs and still others are associated with a variety of distinct gastrointestinal illness [4]. Together, these differences have long suggested that wide genetic diversity exists within this species. In fact, when comparing the genomes of enterohemorrhagic, uropathogenic and commensal strains, one study found that only 39.2% of all predicted proteins were shared by all three strains [5]. This large genetic and phenotypic diversity of *E. coli* emphasizes the need to group these distinct organisms in ways that can be epidemiologically meaningful. Molecular techniques, such as multilocus sequence typing (MLST), have provided epidemiologists with the ability to identify new modes of transmission of infectious agents and detect strain specific outbreaks amongst endemic disease patterns.

UTIs are commonly described as sporadic events caused by poor personal hygiene, sexual intercourse, catheterization and forms of contraception. However, genotypic investigations have revealed that many CA-UTI cases, which appeared to be sporadic, are caused by distinct sets of *E. coli* lineages that may be epidemiologically related [6-8]. If community acquired UTI transmission is spatially driven, we would hypothesize that disease occurrence will cluster spatially by lineage.

In this cross-sectional study, we prospectively collected 1,006 urine isolates from UTI patients for a period of 4 months. These isolates were linked to patient address, as well as medical and demographic data. Using this information, we were able to sequence and identify E. *coli* lineages within our collection and investigate spatial patterning of prevalent *E. coli* lineages causing UTIs in our patient population. This is the first study able to link UTI genotypic data to individual patient data and examine potential for geographic disease clusters. By further understanding how *E. coli* lineages causing CA-UTIs are distributed within a community, we can inherently change our understanding of disease transmission in the context of UTIs, allowing public health specialists to better detect and address local outbreaks and possible environmental exposures.

MATERIALS AND METHODS

Isolate collection

Zuckerberg San Francisco General Hospital (ZSFG) is a large safety-net public hospital and trauma center in San Francisco, that serves an estimated 100,000 patients annually. The ZSFG microbiology laboratory

additionally conducts clinical testing for 15 San Francisco Health Network (SFHN) clinics and a local chronic care facility, located in 14 diverse neighborhoods [34]. We collected all bacteria isolated from clinical urine samples from May 2019 to August 2019 (N=1007) processed at the ZSFG microbiology laboratory.

This study was approved by the University of California, San Francisco, Committee on Human Research. Information regarding dates of specimen collection and medical record numbers (MRN) were used to link isolates to eMR data. In this study, CA-UTI episodes caused by *E. coli* were defined as cases in which urine culture was obtained from an outpatient clinic or emergency department or within 48hrs of inpatient admission and yielded an organisms identified as *E. coli*.
Patient demographic and comorbidity data were extracted from eMRs: patient address, age at time of culture; sex (male or female); Race/Ethnicity (Asian/Pacific Islander, Black, Latinx, White, or Other/ Declined to State); and preferred language spoken (Mandarin and Cantonese, English, Spanish, Other or Not Stated). Comorbidities were evaluated based on previous 5 years of eMR ICD-9 and ICD-10 codes and an unweighted Charleston Comorbidity Index score was calculated (Table 1).

Organism identification and susceptibility testing

Bacterial colonies from ZSFG microbiology laboratory were collected on blood agar purity plates and isolation was additionally confirmed by streaking on MacConkey and Blood Agar Biplates. Isolates were collected after undergoing culture on relevant selective media, further sub-culturing, and having biochemical profiles confirmed by ZSFG clinical microbiology laboratory protocols based on current Clinical and Laboratory Standards Institute (CLSI) guidelines to provide culture-based identification. Isolates were biochemically identified to the species level with API 20E (bioMérieux, Durham, NC) for fermenters or API 20NE for non-enteric bacteria. Indole testing was conducted as secondary confirmation of presumptive *E. coli* at a UC Berkeley laboratory. ZSFG microbiology laboratory performed AST using Microscan WalkAway Gram-negative panel and disk diffusion, with classification of resistance based on CLSI breakpoint standards [9]. The microbiology laboratory classified extended-spectrum beta-lactamase producing *E. coli* (ESBL-*E. coli*) as an *E. coli* strain resistant to ceftazidime or cefotaxime and inhibited by clavulanic acid using broth microdilution, per 2016 CLSI guidelines [9]. Results reported as "intermediate resistance" were considered resistant in this study.

DNA extraction and sequence typing

All bacterial DNA was extracted by freeze-boil method and *E. coli* sequence types (STs) 69, 73, 95, and 131 were identified by a validated multiplex PCR yielding PCR products of expected sizes (Table 2). Gel electrophoresis was used to distinguish unique band sizes to identify *E. coli* sequence types [10].

Statistical analysis

Key demographic and isolate characteristics were summarized with descriptive statistics, including frequencies and percentages for categorical data and mean values with maximum and minimum values for continuous data.

Patient addresses extracted from eMR were geocoded and all spatial analyses were conducted with ArcGIS Pro. All patients without San Francisco residential addresses or who did not meet the criteria of CA-UTI were excluded from analyses. We conducted spatial analyses to identify geographic clusters of

the 4 major pandemic *E. coli* STs within San Francisco county. A kernel density heatmap was created to describe the CA-UTI patient distribution within San Francisco. Patient residential confidentiality was ensured by randomly substituting new point data within a fixed buffer diameter around the original address location. Potential for spatial heterogeneity amongst each of the four lineages was assessed by Global Moran's I testing based on Euclidean distance and inverse distance methodology, such that all patients have at least 1 neighbor.

Cluster identification was conducted through Local Moran's I testing based on Euclidean distance and fixed distances. Bond threshold was determined by iteratively testing distances beginning at the average distance between cases to maximize spatial autocorrelation. Choropleth maps were generated by conducting a spatial join of cluster locations within San Francisco neighborhood boundaries defined in 2006 by the Mayor's Office of Neighborhood Services and colored to visually display the number of cluster hot spots and cluster cold spots of each dominant lineage within San Francisco [11].

RESULTS

Patient demographic characteristics and isolate typing information

Of 551 UPEC isolates in the study, 247 (45%) were identified as pandemic lineages. ST131 was the most common lineage comprising 72 (29%) of the pandemic STs, while contributing the majority (85%) of multi-drug resistant (MDR) isolates among pandemic STs (N=29) and 81% of ESBL isolates (N=22). Among the study population, only 40 isolates (7%) originated from male patients. The median age was 48 with the oldest patient represented being 95 years of age and the youngest at one year. The most common race/ethnicity in our population was Latinx and the most common preferred languages were English, followed by Spanish.

Of the 551 *E. coli* patient isolates, 10 patient addresses could not be geolocated and 19 did not meet CA-UTI inclusion criteria. Additionally, 32 patient addresses were located outside of San Francisco County and excluded from the analysis. The distribution of UTI patient addresses within San Francisco was visualized in a kernel density heat map (figure 1). The highest density of cases captured within our collection are represented in red and yellow. The outcome of the Global Moran's I tests of ST95, ST131, ST69 and ST73 provide evidence of spatial heterogeneity of individual lineages ($p<0.001$, $p<0.001$, $p=0.008$, $p<0.001$, respectively) within San Francisco County. This suggests there is an uneven distribution of various concentrations of each ST within San Francisco, warranting further cluster resolution. Results of Local Moran's I further discern hotspots and coldspots of each of these four STs and demonstrate clustering of each of the four lineages. A choropleth map (figure 2) exhibits the presence of hotspots and coldspots with red and blue color ramps that represent spatial clusters or spatial outliers of each pandemic lineage as detected by Local Moran's I.

DISCUSSION

Geographic information systems (GIS) have been commonly used to analyze and describe the geospatial distribution of many diseases in recent decades and are useful in the control of infectious disease. Understanding disease distribution and the potential of spatial clustering can provide insight into disease transmission, potential exposure sources and disease reservoirs. Community transmission of AMR UTI is a critical public health concern that warrants improved investigations. This study leverages molecular data to characterize the spatial distribution of UTIs, leading to new implications for modes and patterns

of disease transmission. In this study of urine *E. coli* isolates collected consecutively over 4 months, we found that 70% of UTIs diagnosed at a large safety-net healthcare system in San Francisco were caused by *E. coli* and roughly half of those belong to 4 distinct lineages (ST95, ST69, ST131, ST73). These lineages are strongly associated with AMR and are implicated in a large proportion of UTI cases. Lineage ST131, which comprises 29% of our collection, has long been a lineage of concern, as it is strongly associated with ESBL phenotype and MDR. This is consistent with our collection's findings, as ST131 contributes 85% of MDR *E. coli*.[12] Lineage ST95, conversely, has a documented propensity for remaining drug susceptible. [6-8] In our collection, 56% of ST95 isolates were found to be pan susceptible. Thus the geographic distribution and dissemination of these lineages may have major implications for the transmission of AMR CA-UTI.

To date, there is some evidence of spatial clustering of AMR amongst cases of CA-UTI, but no study has established clustering of UPEC lineages. In Brazil, neighborhood-level clusters of fluoroquinolone-resistant *E. coli* causing CA-UTI were identified and in the West of Ireland, geospatial mapping of resistant *E. coli* isolates revealed that a majority of AMR isolates clustered in urban regions [13,14]. These studies focused on how prescribing practices in these areas may be associated with these clusters of resistant phenotypes. However, our work is the first to demonstrate the possibility that dissemination of already resistant lineages may be playing a major role in the distribution of AMR CA-UTI.

Our study is the first to demonstrate spatial heterogeneity in community ST prevalence and is the first to find spatial clusters of CA-UTI. This may suggest that UPEC transmission within a community may occur person to person, or that lineages may disseminate from a handful of point source exposures. Thus, these bacteria may be acquired from contaminated food products or other external sources within the built environment (e.g., water, environment) [15-21]. In fact, in chapter one's systematic review we found that ESBL producing *E. coli* belonging to the lineages same lineages (ST131, ST69, ST73) were found in food sources, companion animals and water sources [15]. Recently, a phylogenetic analysis and plasmid interrogation of ST131, recovered from poultry products, was found to be closely related to ST131 isolated from humans residing in the same region [20].

Our analysis relies on patient residential address to geolocate cases which is limited in its ability to capture disease distribution and transmission as it occurs in workplaces, schools, community venues, residences of close contact and other settings. Additionally, the restriction of our spatial analysis to only patients with residential addresses and/or are not experiencing homelessness is also limiting. This study employed a large sample size and cross-sectional study design which provides an opportunity to assess the prevalence of AMR *E. coli* causing UTI and circulating sequence types important for disease surveillance.

CONCLUSION

The identification of unknown modes of transmission is a key component of molecular epidemiology. Through the use of higher resolution subtyping methods, we can better identity disease clusters, which can contribute to new prevention and intervention strategies. All together, these results suggest that UTI in San Francisco is spatially distributed and that the circulation of UPEC lineages within a geographic region could play a major role in shaping community prevalence of AMR UTI. This finding warrants future studies that prioritize spatial heterogeneity in UPEC lineages within CA-UTI and the investigation of community level risk factors for AMR UTI with special focus on the potential role of the built environment.

	All isolates (551)	ST95 (70)	ST131 (72)	ST69 (53)	ST73 (52)
Age median (max, min) **Separate by gender: Median age of female, male**	48 (1, 95)	52 (20, 95)	53 (17, 89)	36 (3, 88)	40 (6, 75)
Sex (male)	40 (7.3%)	7 (10%)	6 (8.3%)	3 (5.7%)	6 (11.5%)
Race/ Ethnicity					
White	57 (10.3%)	3 (4.3%)	15 (20.8%)	3 (5.7%)	5 (9.6%)
Black	36 (6.5%)	6 (8.6%)	8 (11.1%)	2 (3.8%)	3 (5.8%)
Asian/Pacific Islander	65 (11.8%)	20 (28.6%)	5 (6.9%)	4 (7.5%)	8 (15.4%)
Latinx	200 (36.3%)	25 (35.7%)	19 (26.4%)	21 (39.6%)	19 (36.5%)
Other/ Declined to state	358 (64.9%)	54 (77.1%)	47 (65.2%)	30 (56.6%)	35 (67.3%)
Preferred Language					
English	205 (37.2%)	32 (45.7%)	36 (50.0%)	13 (24.5%)	19 (36.5%)
Spanish	140 (25.4%)	18 (25.7%)	13 (18.1%)	17 (32.1%)	12 (23.1%)
Mandarin & Cantonese	22 (4.0%)	5 (7.1%)	2 (2.8%)	1 (1.9%)	3 (5.8%)
Other	19 (3.4%)	2 (2.9%)	1 (1.4%)	3 (5.7%)	2 (3.8%)
Not Stated	165 (29.9%)	13 (18.6%)	20 (27.8%)	19 (35.8%)	16 (30.8%)
Previous UTI	43 (7.8%)	8 (11.4%)	13 (18.1%)	1 (1.9%)	4 (7.7%)
Recurrent UTI	18 (3.3%)	1 (1.4%)	8 (11.1%)	0 (0%)	2 (3.8%)
Co-morbidities					
Diabetes	13 (2.4%)	4 (5.7%)	2 (2.8%)	0 (0%)	1 (1.9%)
Prior Antibiotics (6 mo.)	33 (6.0%)	4 (5.7%)	10 (13.9%)	1 (1.9%)	4 (7.7%)
Malignancy	17 (3.1%)	0 (0%)	8 (11.1%)	0 (0%)	3 (5.8%)
CCI* mean (SD)	3.44 (1.13)	2.63 (2.26)	3.23 (1.01)	3.70 (1.03)	2.50 (2.52)

Table 1. Demographic and health characteristics of patients with UPEC infection by dominant strain type. Patient characteristics were extracted from eMRs: age at time of culture; sex (male or female); Race/Ethnicity (Asian/Pacific Islander, Black, Latinx, White, or Other/ Declined to State); and preferred language spoken (Mandarin and Cantonese, English, Spanish, Other or Not Stated). Comorbidities were evaluated using 5 years of eMR ICD-9 and ICD-10 codes.

*Unweighted Charleston Comorbidity Index score, mild with CCI scores 1–2; moderate with CCI scores of 3–4; and severe, with CCI scores ≥5

Primer	Sequence Type	Sequence (5'-3')	Expected product size (bp)
ST69_for	69	ATCTGGAGGCAACAAGCATA	104
ST69_rev		AGAGAAAGGGCGTTCAGAAT	
ST95_for	95	ACTAATCAGGATGGCGAGAC	200
ST95_rev		ATCACGCCCATTAATCCAGT	
ST131_for	131	GACTGCATTTCGTCGCCATA	310
ST131_rev		CCGGCGGCATCATAATGAAA	
ST73_for	73	TGGTTTTACCATTTTGTCGGA	490
ST73_rev		GGAAATCGTTGATGTTGGCT	

Table 2. Primer sequences used in the multiplex PCR assay for the detection of dominant UPEC Strain Types. All primer sequences and assay specifications were referenced from Doumith et. al 2015

Dominate Strain Type	episodes caused by susceptible urine *E. coli*	episodes caused by antimicrobial resistant					
		Ampicillin	Nitrofurantoin	Trimethoprim-sulfamethoxazole	Fluoroquinolones	ESBL	MDR
ST95	39 (56%)	23 (33%)	0 (%)	38 (53%)	1 (1%)	0 (%)	1 (1%)
ST131	13 (18%)	55 (75%)	2 (3%)	38 (53%)	34 (47%)	22 (31%)	29 (40%)
ST69	13 (18%)	36 (68%)	0 (%)	30 (57%)	3 (6%)	4 (8%)	0 (0%)
ST73	17 (33%)	32 (62%)	0 (%)	13 (25%)	3 (6%)	1 (2%)	4 (8%)

Table 3. Antimicrobial Susceptibility by dominant strain type. Antimicrobial susceptibility testing was performed with Microscan and disk diffusion tests, and ESBL status was confirmed, with reports of resistance based on CLSI breakpoint guidelines. MDR is defined as resistant to at least one agent in ≥ 3 classes of antibiotics

Figure 1: Distribution of CA-UTI within San Francisco caused by *E. coli*. Heat map was created using magnitude-per-unit area from point features using a kernel function within the kernel density tool in ArcGIS pro.

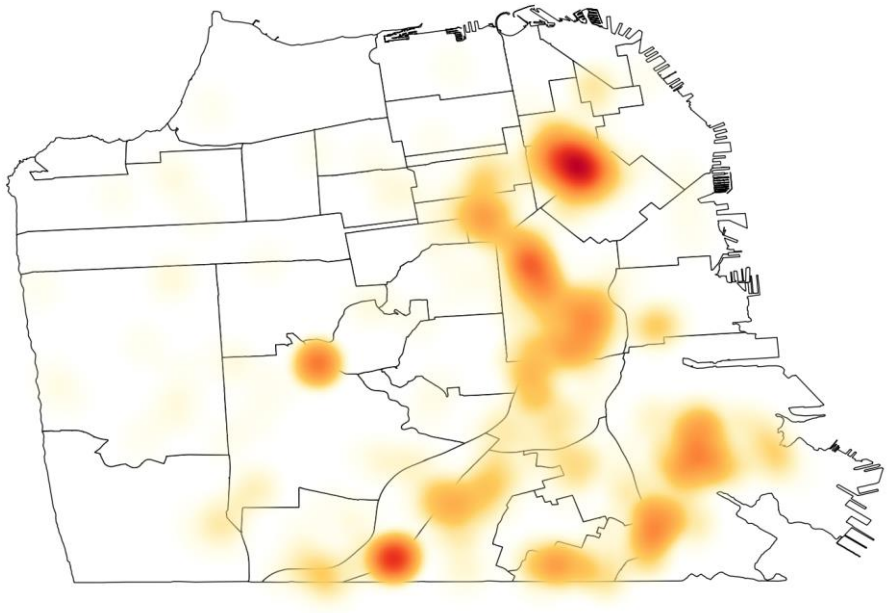
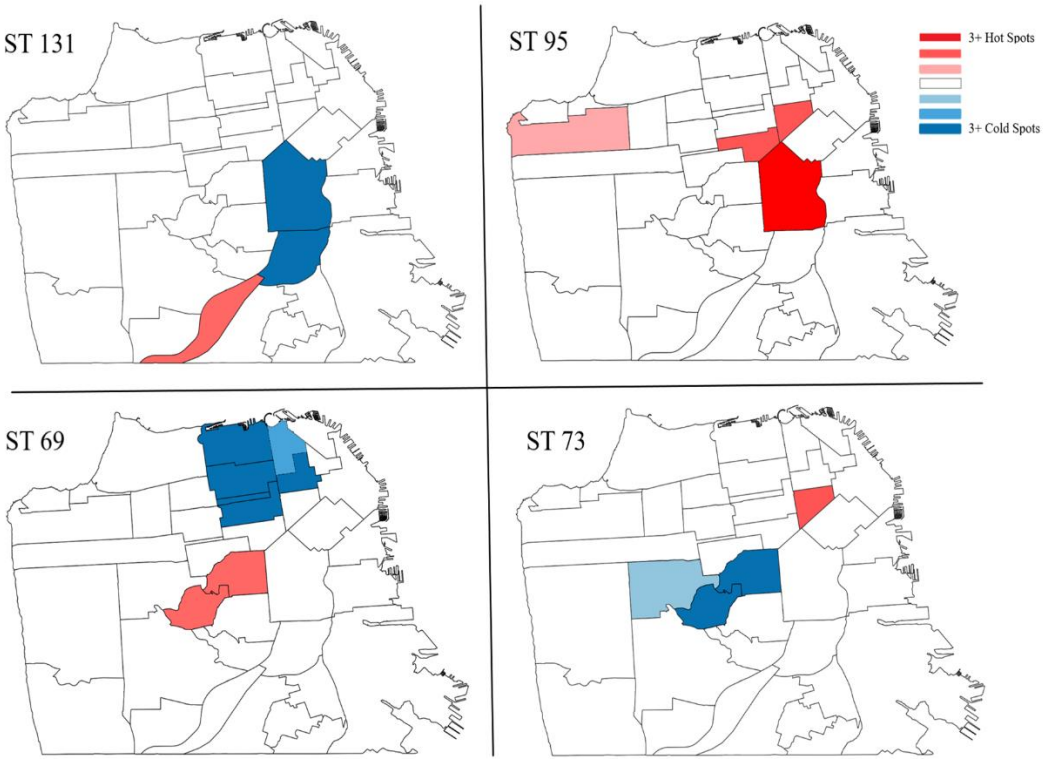


Figure 2: Distribution of spatial clusters of dominant lineages of UPEC within San Francisco
Clusters were detected using local Moran's I applying Euclidian distances. Clusters identified were aggregated to neighborhood features, the sum of which are display in a choropleth map. Red shades denote number of clusters identified and blue shades indicate number of outliers detected in each neighborhood.



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CONCLUDING REMARKS AND FUTURE DIRECTIONS

The application of molecular and microbiology tools within the field of epidemiology can be used to provide the necessary resolution to reveal disease patterns, identify novel risk factors and discover potential targets for interventions and diagnostic development. Throughout this dissertation, these methods were used to deepen our understanding of antimicrobial resistance within the context of community acquired UTI (CA-UTI). This work begins with an investigation of patient risk factors for extended spectrum beta-lactamase producing (ESBL) E. coli causing CA-UTI by systematic literature review (Chapter 1). It continues with an investigation of genetic risk factors for ESBL co-resistance using the whole genome sequences of 557 UTI patient isolates (Chapter 2). The final chapter of this body of work represents the creation of a cross sectional study, which prospectively collected patient urine isolates from 1006 patients. These isolates were genotyped and linked to patient medical records. Using this uniquely large and complete collection, we conducted a spatial investigation of E. coli genotypic clustering as a means to better understand geographic distribution of E. coli lineages associated with resistance and gain insight into transmission and dissemination.

Chapter 1. In this study, we found that factors such as previous UTI episodes and recurrent UTI may represent risk factors for drug-resistant UTI, but those studies reviewed largely lacked the proper comparison groups to identify risk factors for UTI caused by ESBL-producing *E. coli*. Such observations may result from our current lack of precise understanding of mode of transmission of CA-UTI, as the majority of studies did not investigate community-level and environmental risk factors. Furthermore, this review found compelling evidence of the presence of ESBL producing pandemic UPEC lineages that have been implicated in human cases of CA-UTI in food animals, companion animals and other environmental sources. These results may suggest that there are in fact point sources of human exposure to these pathogens.

Chapter 2. This work provides a regional description of prevalent MDR UPEC lineages, phenotypic co-resistance profiles, and resistance determinants in uropathogenic *E. coli* resistant to third generation cephalosporins. This study highlights targets for improved antimicrobial resistance surveillance and helps identify genes underlying the cephalosporin resistant phenotype within clinical isolates in California. By elucidating the specific β-lactamase genes present in these suspected MDR organisms, we increase our understanding of associated co-resistance profiles. Our results suggest the co-occurrence of blaCTX-M-15/blaOXA-1/aac(6')-Ib-cr genes and the occurrence of blaCTX-M-55, constitutes an important risk factor for MDR. Identification of these markers, in addition to ESBL phenotype could inform empiric treatment decisions, including targeted carbapenem sparing strategies, to not only promote antibiotic stewardship, but improve treatment outcomes.

Chapter 3. We generated a dataset of patient urine isolates and linked it with clinical and demographic information we extracted from patient medical records. We genotypically and phenotypically characterized over 1000 isolates in the course of this work. This collection is currently in the process of being fully characterized through whole genome sequencing. Once sequencing is complete, we will be able to more deeply investigate the relatedness of clinical and environmental samples, the relatedness of disease clusters, as well as provide a platform for many other investigations. Our finding of the existence of lineage specific disease clusters provides the basis of a mediation analysis that will quantify the role

that circulating lineages play in the relationship between community characteristics and AMR in that community.

To combat AMR threats such as ESBL and carbapenem resistance in Gram-negative bacteria, like *E. coli*, this work focuses on addressing four CDC and WHO strategies; 1) infection control and prevention, 2) improved surveillance of AMR, 3) stewardship promotion and improved prescribing practices and 4) development of AMR diagnostics and new antimicrobial agents. Chapter 1 investigates *E. coli* lineage as a risk factor of ESBL and suggests a new strategy for AMR related surveillance. Chapter 2 focuses on improvements to prescribing practices and identifies targets for AMR diagnostic development. Through our collaboration with industry members, this chapter lays the foundation for their work in developing these technologies. While Chapter 3 furthers our understanding of UTI transmission, which can contribute significantly to infection control and prevention of these AMR infections. Altogether this body of work hopes to contribute to a global effort to combat the rising threat of AMR.