Risk factors for community acquired urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing *Escherichia coli* in children: A case control study

Background

Extended-spectrum β lactamases (ESBLs) are β-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam aztreonam. Thus ESBLs confer resistance to these antibiotics and related oxyimino-β lactams [1, 2].

Frequent use of antibiotics in humans and animals has caused an increase in resistance rate of Gram negative (G-ve) bacteria in recent years. There is an increasing resistance reported in G-ve bacteria to commonly used antibiotics in treatment of urinary tract infections (UTI) such as trimethoprim-sulfamethoxazole, fluoroquinolones or other standard antibiotics [3]. However, in recent years, there has been an emergence of G-ve bacteria that produce ESBLs [4]. Initially these organisms were found among patients who were hospitalized or exposed to antibiotic treatment. However, an increasing number of patients with community acquired resistant infections has been noted. These patients were mainly adults and their infections with ESBL producing organisms was associated with increased mortality and morbidity [5-7].

The most common organisms that were associated with community acquired ESBL infections are *Escherichia coli* and *Klebsiella* species. These same organisms cause acute uncomplicated UTI, thus the treatment of UTI is becoming more challenging. The identification of risk factors for antimicrobial resistance may improve empirical treatment of UTI. In adult studies, reported risk factors for community acquired UTI due to ESBL-producing G-ve bacteria include older age, female sex, diabetes mellitus, recurrent UTI, invasive urological procedures, previous urine catheters and prior use of antibiotics [8, 9]. It has also been shown that previous use of antibiotics including cephalosporins, penicillins or fluoroquinolones was associated with ESBL-producing G-ve infections [8, 9].

Studies of the risk factors associated with ESBL-producing G-ve infections in children have mainly focused on hospitalized children [10, 11]. However, few recent studies of community acquired ESBL infections in children are published. Topaloglu et al found that having an underlying disease and hospitalization within the last 3 months were potential risk factor for infection with ESBL-producing *E coli* and *Klebsiella* in children [12]. Previous exposures to antibiotics and young age (< 1 year) have also been demonstrated to be risk factors in additional studies [13]. However, these studies were done in countries with known high prevalence of ESBL infections or in medical centers that use cephalosporins in antibiotic prophylaxis. Recent studies have shown that the rates of ESBL infections in children in the USA are increasing [14]. However, the risk factors of ESBL-producing bacteria in the community acquired UTI in children in the USA remain unclear.

In recent years, an increasing number of children with community acquired (CA)-UTI due to ESBL-producing organisms, especially *E. coli*, has been observed at our institution. The primary aim of this study was to determine the frequency of CA-UTIs caused by ESBL-producing bacterial pathogens in children seen at Children's Hospital of Michigan during 2012-2016 and investigate the characteristics of children to determine the risk factors associated with these infections.

Patients and Methods

Children who presented to our hospital with CA-UTI due to ESBL-producing *E. coli* during the period January 2012 - January 2016 were included in the study. The Children's Hospital of Michigan is a 220-bed tertiary care center in Detroit, Michigan. Urine cultures that were positive for ESBL-

producing *E. coli* were identified from the records of the University Microbiology Laboratory of the Detroit Medical Center. A control group consisting of children with UTI caused by non-ESBL-producing *E. coli* was included. Patients in the control group were matched by age, gender, and year of the CA-UTI due ESBL-producing *E. coli* group.

Exclusion criteria included positive urine cultures >72 hours after hospitalization, patients with long term care facility stay within the preceding 3 months, postoperative infections within 10 days of surgery, and asymptomatic bacteriuria.

Each urine culture was included once in the study. If more than one positive ESBL-producing *E. coli* urine culture was present, the last clinical record with the least missing data was included. Positive urine culture was defined according to the method of collection of the urine sample. Bag specimens were not included in the analysis. In midstream specimens of urine, UTI was defined as a positive urine culture $\geq 10^5$ CFU/mL or a positive urine culture (10^4 - 10^5 CFU/mL) with pyuria of ≥10 leukocytes per high power field. In specimens obtained through by bladder catheterization, growth of 10^4 - 10^5 CFU/mL was defined as UTI.

Medical records of patients with UTI caused by ESBL-producing and non-ESBL producing *E coli* were reviewed to obtain information on demographic characteristics, history of hospital visits, clinical findings, urine culture pathogen its antimicrobial susceptibilities, laboratory and imaging studies, comorbidities, treatment modalities, hospital course, complications, and outcome. Information was collected and analyzed for the following potential risk factors for ESBL infection: history of previous UTI, anatomic abnormalities of the urinary tract, antibiotic usage in the past 3 months, previous hospitalizations, intensive care unit stay, surgeries, underlying neurologic abnormalities such as spina bifida or neurogenic bladder, previous infections, history of infection with ESBL-producing bacteria or other resistant bacteria, and intermittent urinary bladder catheterization.

Microbiological identification

Each urine specimen was collected in urine collection tube containing borate, then a sample was plated on BAP and MAC agar plates using a 1 microliter sterile loop. Plates are incubated at 35 C overnight (18-24 h). Colonies were counted and multiplied by 1000 to report the CFU/ml of urine.

Colonies resembling *E. coli* (Gram negative bacilli, lactose fermenter) were processed in Phoenix automated bacterial ID and antibiotic susceptibility instruments. BD Phoenix identification and susceptibility combo panels (NMIC/ID303) were used for ID and susceptibility. ESBL status was determined by resistance marker testing included in the panel.

Statistical analysis:

Data on different clinical variables and frequencies were analyzed using SPSS version 20. A non-parametric Fisher's Exact test was employed to examine potential differences between study groups on categorical variables. An independent sample T-test will examine mean differences between study groups. Variables found from univariate factors described above were entered into a binary logistic regression equation to find the best predictors of acquiring infection with ESBL positive bacteria. A p value of <0.05 was considered statistically significant.

Results

A total of 111 cases of community acquired ESBL producing *E. coli* UTI were identified during 2012-2016. A total of 103 cases of community acquired non-ESBL producing *E. coli* UTI were matched by age, gender, and year of the study period. No matching cases were found for 8 of the ESBL-positive cases, accounting for a slight imbalance in the groups.

When compared to the total number of UTIs diagnosed at our hospital during the study years, the incidence of ESBL-producing *E. coli* UTIs accounted for 11% of all UTIs seen in 2012, 7% during 2013, 9% in 2014, 7% in 2015 then increased to 15% in 2016.

During the study period, patients with community acquired ESBL producing *E. coli* UTI were predominantly female (84%). The age range was 3 months to 17 years with a median age of 4 years. The racial distribution was of the ESBL-positive group was predominantly African American (32%) and Middle Eastern (31%) followed by Caucasian (17%) and Hispanic (16%). The racial distribution of the control group was predominantly African American (49%), followed by Hispanic (22%), Caucasian (17%), and Middle Eastern (10%). Middle Eastern ethnicity was significantly more prevalent in the ESBL group than in the control group ($p<0.001$).

Clinical presentation of ESBL producing and non-ESBL producing *E. coli* UTI groups are presented in Table 1. No significant difference was found in the frequency of fever, dysuria, frequency, enuresis or abdominal pain. The ESBL-positive group was found to have statistically significant lower incidence in flank pain (4.6% ESBL vs 15.5% non-ESBL; $p=0.010$). No significant difference was found in WBC, hemoglobin/hematocrit, platelet count, BUN, or CRP at presentation. ESBL producing group was found to have statistically significant lower mean creatinine at presentation (0.48 ESBL vs 1.57 non-ESBL; $p=0.028$).

Antibiotic susceptibility results of the two groups are presented in Table 2. ESBL-producing *E. coli* strains were universally susceptible to amikacin, meropenem, and imipenem. Only two strains (2%) in the ESBL-producing group were resistant to ertapenem and one (1%) to nitrofurantoin. Resistance to β-lactam antibiotics was nearly universal by minimum inhibitory concentration (MIC): cefepime 88%, cefoxitin 86%, ceftriaxone 100%, cefazolin 100%. However, given confirmed presence of ESBL in these isolates, all ESBL strains are treated as cephalosporin-resistant in clinical practice despite in vitro sensitivity. ESBL-producing *E. coli* strains were noted to be more resistant to gentamicin (36% ESBL vs 5% non-ESBL), tobramycin (39% ESBL vs 2% non-ESBL), and trimethoprim/sulfamethoxazole (72% ESBL vs 25% non-ESBL). Resistance to ciprofloxacin was higher in ESBL (73%) compared to non-ESBL strains (5%).

ESBL producing group was noted to have increased prevalence of pre-existing medical conditions (46% ESBL vs 10% non-ESBL) with higher rates of myelomenigocele (10% ESBL vs 6% non-ESBL) and cerebral palsy (3% ESBL vs 0% non-ESBL). However, univariate analysis of risk factors associated these underlying medical conditions did not show statistical significance. These risk factors included functional abnormalities including neurogenic bladder, voiding dysfunction, concurrent neurogenic bladder and voiding dysfunction, constipation, use of intra-urinary device (clean intermittent catherization, ureteral stent placement), presence of GU abnormalities, intraurinary tract intervention or prior surgery in the last 3 months (excluding surgery in the last 10 days). Additionally, immunosuppressed status was not statistically significant. The only risk factor associated with an underlying medical condition that was statistically significant was presence of vesicoureteral reflux (VUR) (20.9% ESBL vs 6% non-ESBL; $p=0.002$).

Univariate analysis of risk factors for community acquired ESBL producing *E. coli* UTI is shown in Table 3. Statistically significant risk factors found in univariate analysis included prior antibiotic usage in the last 3 months (54.5% ESBL vs 14.6% non-ESBL, $p<0.001$), prior β-lactam use in the

last 3 months (30.9% ESBL vs 8.8% non-ESBL; $p<0.001$), presence of VUR (20.9% VUR vs 5.9% non-ESBL; $p=0.002$), prior UTI in the last 3 months (24% ESBL vs 6% non-ESBL; p=0.002), prior hospitalization in last 3 months (24.5% ESBL vs 10.1% non-ESBL; p=0.007), Middle Eastern race (30.6% ESBL vs 9.8% non-ESBL; $p<0.001$).

History of antibiotic usage in the last 3 months ($p=0.001$, OR 4.17, 95% CI 1.85-9.09) and Middle Eastern race ($p<0.001$, OR 4.00, 95% CI 1.69-9.09) remained significant in multivariate analysis. Prior UTI ($p=0.097$), VUR ($p=0.152$), and presence of GU abnormalities ($p=0.065$) were not significant.

Discussion

While the presence of ESBL-producing *E. coli* has been described as a predominantly healthcare associated infection since the late 1980s [15], increasing incidence of community acquired infections since the turn of the century have been reported. Nearly half of ESBL-producing *E. coli* strains isolated from a hospital in Seville, Spain between 2001 and 2002 were defined as community acquired (no hospitalization/nursing home stay in last 1 year, cultured <48 hours after admission). Known risk factors for ESBL-production *E. coli* were reported to be diabetes mellitus, recent use of fluoroquinolones and hospital admission in the prior year. [7]. In a recent survey of 5 centers across the United States from 2009-2010 (New York City, Detroit, Pittsburg, Iowa City, San Antonio) more than one-third of community-onset ESBL-producing *E. coli* infection were community associated [16].

There is no consensus on possible risk factors for community associated ESBL-producing *E. coli* infection in pediatric patients in the limited data available. A recent retrospective observation study from 2015-2016 of pediatric patients in Toledo, Spain proposed male sex, hospitalization within last 30 days, VUR, and urological pathology as possible risk factors [17]. In contrast, underlying neurological diseases, developmental delay, recurrent UTI, recent hospitalization, and prior antibiotic use were proposed as risk factors from a 2002-2006 study in Taiwan [18]. In a study of patients from 2004-2006 in Turkey, Topaloglu et al proposed underlying disease and hospitalization/infection/antibiotic use in the prior 3 months as possible risk factors for ESBL-producing UTI in children [12].

A possible explanation for this divergence of risk factors is due to the regional variation of the study populations. ESBL prevalence varies by geographic location with the highest rates of >50% of *E. coli* isolates in SE Asia (China, India), followed by 25-50% in the Middle East (Turkey, Egypt, Iraq, Iran), and 20-25% in the United States and Western Europe (UK, France, Spain, Portugal). The trend of higher rates of ESBL prevalence is reflected in the fecal colonization of the healthy individuals with ESBL-producing *Enterobacteriaceae* (46% West Pacific, 22% SE Asia, and 22% Africa vs 4% Europe and 2% Americas) [19]. Additionally, international travel, particularly to India and SE Asia, has been found to be a risk factor for development of community onset ESBL-producing *E. coli* infection [20].

Indeed, the statistically significant higher proportion of patients of Middle Eastern origin with ESBL-producing E. coli UTI compared to other ethnic groups (Caucasian, African American, and Hispanic) in our study suggests ethnic background may be an important risk factor for development of community onset ESBL-producing *E. coli* UTI in children. The majority of these patients resided in the city of Dearborn, MI. Dearborn that has the largest proportion of Arab Americans in the United States. The increased risk in this group may be reflective of possible variations in dietary habits, international travel, and fecal colonization. Indeed, travel to the Middle East and North Africa was found to increase the risk of fecal colonization with ESBL-producing *Enterobacteriaceae* [21]. However, prevalence of fecal carriage greater than 3 months is uncommon [22] and further

investigation into possible risk factors which may contribute to higher risk of development of community associated ESBL-producing *E. coli* UTI in this ethnic group is warranted.

The second major risk factor we found in our study population to be statistically significant in multivariate analysis was prior use of antibiotics within the last 3 months. Additionally, the subset of prior β-lactam antibiotic use was demonstrated to statistically increase the risk of ESBL-producing *E. coli* UTI in univariate analysis. Prior antibiotic use has been suggested as a risk factor in prior studies in both pediatric [12, 18] and adult patients [8, 9, 23]. Prolonged antibiotic use has been demonstrated to increase the prevalence of *Enterobacteriaceae* resistant to ampicillin and trimethoprim-sulfamethoxazole in the GI tract of the CF population [24]. Therefore, prior exposure to antibiotics, particularly β-lactam antibiotics, may induce selection of ESBL-producing *E. coli* in the gastrointestinal (GI) tract. As intestinal colonization generally precedes onset of infection by gram-negative pathogens [25], the subsequent increased colonization of ESBL-producing *E. coli* in the GI tract may lead to increased risk of ESBL-producing *E. coli* UTI.

In our study patients, prior UTI in the last 3 months, hospitalization in the last 3 months, and presence of VUR were demonstrated to be statistically significant risk factors in univariate analysis but not significant in in multivariate analysis. However, these risk factors are likely associated with the risk factor of prior antibiotic use thereby lowering their significance in multivariate analysis. Patients with prior UTI and a significant proportion of hospitalized patients would have been treated with antibiotics. The presence of VUR increases exposure to antibiotics through both increased risk for UTI and use of antibiotic prophylaxis. Indeed, 11/60 patients exposed to prior antibiotic use in the ESBL producing group were receiving trimethoprim-sulfamethoxazole prophylaxis for VUR.

As the genes for ESBL and resistance genes for other classes of antibiotics are often encoded together on transferable plasmids [26], ESBL producing organisms are often multidrug-resistant (MDR). The higher rates of coresistance to non β-lactam antibiotics (trimethoprim-sulfamethoxazole, aminoglycosides, and quinolones) in the ESBL *E. coli* specimens when compared to non-ESBL specimens is consistent with previously observed resistance patterns in ESBL-producing organisms [15, 27]. Higher rates of resistance of ESBL-producing *E. coli* to non β-lactam antibiotics has been reported throughout the world including Iran, Turkey, Spain, and Israel [8, 9, 28, 29]. Therefore, exposure to non β-lactam classes of antibiotics, including trimethoprim-sulfamethoxazole used in the prophylaxis of VUR, can induce selection of ESBL-producing *E. coli* in the GI tract. Judicious use of antibiotics in the community is necessary to stem the increasing prevalence of community acquired ESBL-producing *E. coli* strains. Additionally, the inherent MDR nature of ESBL-producing *Enterobacteriaceae* will pose a challenge in antibiotic treatment of these infections.

While underlying medical conditions, including neurological conditions, were suggested as possible risk factors in prior studies, no such association was found in our study in univariate analysis [18]. There was a preponderance of myelomeningocele in the ESBL producing group (10% ESBL vs 5.9% non-ESBL). However, neurological abnormalities associated with myelomeningocele (neurogenic bladder, voiding dysfunction, concurrent neurogenic bladder and voiding dysfunction, use of intraurinary device such as clean intermittent catherization or ureteral stent placement) were not found to be significant on univariate analysis.

Conclusions

Similar to the previous emergence of community associated methicillin-resistant S. aureus (CA-MRSA), the emergence of community associated ESBL-producing *E. coli* as a pathogen presents challenges not only in diagnosis and treatment, but also in infection control. Currently, there is limited data available on possible risk factors for the development of community associated ESBL-producing *E. coli* UTI. In our study, prior antibiotic use in the last 3 months and Middle Eastern

race were found to be statistically significant in multivariate analysis. Both of these risk factors are associated with increased risk of fecal colonization with drug resistant organisms, including ESBL-producing *Enterobacteriaceae*.

The increased risk of community associated ESBL-producing *E. coli* among children of Middle Eastern ethnicity suggests that history of international travel or contact with international travelers should be obtained when evaluating a child for possible community acquired ESBL infection. Prior antibiotic usage as a risk factor reinforces the need for judicious use of antibiotics, particularly β-lactam antibiotics, to reduce further spread of ESBL-producing *E. coli* infections. Additionally, 18% of patients with prior antibiotic use in the ESBL-producing *E. coli* UTI group were on long term Bactrim prophylaxis for VUR. Further study into the effects of antibiotic prophylaxis on the prevalence ESBL-producing *E. coli* UTI infection is warranted as this practice may paradoxically increase the prevalence of ESBL-producing UTI in a patient population already prone to recurrent UTIs.

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