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A Retrospective Cohort Study Of High Prevalence Of St131 Among Extended Spectrum B-Lactamase Producing E. Coli Among Inpatients In The Metropolitan Detroit Area

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A RETROSPECTIVE COHORT STUDY OF HIGH PREVALENCE OF ST131 AMONG EXTENDED SPECTRUM β-LACTAMASE PRODUCING *E. COLI* AMONG INPATIENTS IN THE METROPOLITAN DETROIT AREA

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DEDICATION

This work is dedicated to my parents, professors and siblings, for being strong and believing in me.

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CHAPTER 1 "*ESCHERICHIA COLI*"

1.1 INTRODUCTION

Escherichia coli (*E.coli*) (1) are Gram-negative, anaerobic bacilli, belonging to the family Enterobacteriaceae. *E.coli are* commonly found in the lower intestine of warm- blooded species. Most strains of *E.coli* typically do not cause symptomatic disease, but some strains of E.coli are virulent and cause diseases ranging from brief diarrhea to life threatening forms of kidney failure. In patients with immunosuppression, both pathogenic and nonpathogenic *E.coli* strains can cause intestinal infections.

E.coli infections are the most common cause of urinary tract infection (UTI) (2, 3) and also can be an etiologic agent causing gastroenteritis, neonatal meningitis, septicemia and bacteremia (3). A major route of transmission for *E.coli* infections is through contaminated food and water (3). Young children and older adults are most commonly infected; however healthy people typically recover from the infection within 5-7 days. Apart from the harmful effects, *E.coli* have beneficial functions too. For example, they help in digestion and absorption of food, manage waste material and produce vitamin K_2 (5).

1.2 DIVERSITY

E.coli are extensively diverse bacteria in terms of both phenotype and genotype. There are more than 700 serotypes of *E.coli* that have been classified (6,7). The common classification of *E.coli* is based on the serotype present, which is further based on the surface antigens combinations i.e. O antigen (part of lipopolysaccharide layer), H antigen (flagellin) and K antigen (capsule). A total of 173 O antigen, 80 K antigen and 56 H antigens have been identified, resulting in numerous combinations of subdivisions (6). O antigen is easily detectable and is the main marker used to identify virulent *E.coli* species (7). Based on this classification, there are two groups with common combinations of serotypes (a) diarrhoeal disease serotypes and (b) extraintestinal serotypes (6). Furthermore, diarrhoeal diseases serotypes are divided into four subclasses according to the virulence factor i.e. (a) Enterotoxigenic *E. coli* (ETEC), (b) Enteroinvasive *E.*coli (EIEC), (c) verotoxin (Shiga-like toxin) or Enterohaemorrhagic *E. coli* (VETC or EHEC) and (d) Enteropathogenic *E.coli* (EPEC). These subclasses of bacteria use different mechanisms to cause virulence in hosts (8).

E.coli also have been classified into four additional groups through phylogenetic analyses. The phylogenetic groups are A, B1, B2 and D (9). The pathogenic groups of *E.coli* mostly belong to the B2 phylogenetic group and, to a lesser extent, the D phylogenetic group. In contrast, commensal *E.coli* mostly belong to group A (10).

1.3 ROLE IN DISEASE

Although *E.coli* is considered as commensal, largely nonpathogenic bacteria, all of the strains of *E.coli* are not harmless. They can lead to disease from intestinal infection to fatal disease. Because of the diversity among the strains of *E.coli*, they can be categorized either into intestinal pathogenic *E.coli* or extraintestinal pathogenic *E.coli* (ExPEC) (11). ExPEC possess a unique ability to cause infections outside the patient's intestinal tract. They possess distinctive virulence mechanisms and transmission pathways (12). The most common symptom of intestinal *E.coli* is diarrhea. ExPEC is further classified into Uropathogenic *E.coli* (UPEC). UPEC causes neonatal meningitis and septicemia (11). These strains generally belong to the B2 and the D pathogenic groups. Strains having virulent genes (VG) are mostly from the B2 pathogenic class, and rarely from the D class.

Urinary tract infections (UTI) are one of the most common infections in patients worldwide (2). In addition, *E.coli* is also affects the other sites like respiratory tract, bones and joints, extra-abdominal sites and skin and soft tissue: but these sites are less likely to be infected by *E.coli* (13).

1.4 EPIDEMIOLOGY AND TREATMENT FAILURE

After *Staphylococcus aureus*, *E.coli* is the second most common source of clinical infections that accounts almost 17.3% of infections that require hospitalization. Majorly responsible for bloodstream infections and UTI, approximately 50% are hospital-acquired infections (HAI) and 80-90% are community-acquired infections (14). The major reason for the increasing prevalence of *E.coli* infections is the antimicrobial resistance.

1.4.1 ANTIMICROBIAL RESISTANCE

Emergence of antibiotic resistance has become a serious issue all over the world, threatening public health and limiting drug choices to cure infections. Several studies have tried to determine the mechanism or the reason behind multidrug resistance.

FLOUROQUNILONES RESISTANCE

Quinolones have always been a preferable class of drugs due to their broad spectrum and few side effects. Extensive use of the quinolones has lead to the evolution of resistance through selective pressure. Several different mechanisms for this emergent resistance have been proposed.

One of the potential mechanisms of resistance is the genetic mutation of the gene encoding DNA gyrase (gyrA) and topoisomerase (parC). DNA gyrase and topoisomerase act as drug targets. Investigations by Lautenbach et.al suggested that there is at least one type of gyrA mutation in fluoroquinolone resistant bacteria (15). They found no isolate with parC mutation without gyrA mutation

and they concluded that nalidixic acid could act as marker for this type of mutation. However, this study had some limitations. The authors did not consider the strain diversity and, furthermore, the study was limited to only one type of common mutation resistance mechanism.

Another mechanism of resistance is the over-expression of efflux pumps in bacteria like AcAB-TolC (15-17). Comprehensive research by Singh et.al suggested three relationships between efflux pumps and quinolones. One was the exponential increase in the susceptibility to fluoroquinolones when there is depletion in efflux pumps. Second was the over expression of efflux pumps which can mediate the early stages of development of resistance. Lastly, the emergence of resistance can be delayed by depleting the efflux pumps (17). The presence of organic solvent tolerance can also be another reason for the resistance, but this mechanism is only seen against chloramphenicol (15).

1.5 CLONAL DISTRIBUTION

Diversity and distribution of *E. coli* clones (i.e. sub-species strains differentiated by genetic polymorphisms) vary according to the patient characteristics, type of infection, severity and resistance profiles (18). DNA profiling has helped to understand the *E.*coli outbreak and advanced international studies have suggested the predominance of several sequence types (STs) ST131, ST95, ST73, ST127, and ST69 (19). According to the research done by Banerjee et.al, 47 different strain types were found among 331 isolates, and among them the most common ones were ST131, ST95, ST73 and ST69 (27). The distribution varied by site of infection, age group, and association with health care and community onset infections. Among these five most common strain types, ST131 was the most prominent one and was represented exclusively by its subclone H30. The authors also commented specifically on resistant phenotypes; even in that profile ST131 were highly prevalent followed by ST69.

1.6 EMERGENCE AND DOMINANCE OF SEQUENCE TYPE ST131 *ESCHERICHIA COLI*

In the context of increasing resistance against antimicrobials, the ST131 clone has recently become the leading clone among all STs of *E.coli*. ST131, defined as multi-locus sequence type (MLST) 131, belongs mostly to the B2 phylogenetic subgroup I and exhibits a specific O-25 type (20). ST131 is gaining importance because of its phylogenetic background, virulence factors and highly multi-drug resistant characteristics. Therefore, the high-virulence potential no doubt has been suspected to be an important contributing factor for the spread of ST131, but the presence of resistance genes is an additional factor leading to failure of antimicrobial treatment.

The association of ST131 *E.coli* with antibiotic resistance was initially reported with ESBL producing *E.coli,* among serogroup O25b:H4 (13). However, its prevalence has also been recorded in non-ESBL producing *E.coli* (21). Extended spectrum β-lactamase (ESBL) is a rapidly emerging class of β-lactamase. ESBL hydrolyzes the β-lactam ring present in the antibiotics by producing β-lactamases. ESBL are generally plasmid mediated, driven by TEM and SHV genes. Amino acid substitution leads to mutation around the active site of β-lactamases. These mutations result in the development of resistance mechanisms not only against penicillin, 1st, 2ndand 3rd generations of cephalosporin but also against aminoglycosides and polymyxins (22). Hence, these mutations extend the spectrum of β-lactams and further limit the available treatment options. In

addition to TEM and SHV ESBL, there are other types of ESBL that are widely distributed globally. Increasing production of ESBL production in *E.coli* and resistance to additional antimicrobial agents, especially fluoroquinolones, is a distinctive feature of the ST131 clone (13).

One type of ESBL that is extensively present worldwide is the CTX-M-ESBL (22, 23). Studies have suggested that the sudden increase of CTX-M producing ESBL *E.coli* is due to ST131, which may have had increased clonal expansion as compared to the other non-ST131(1). A cohort study was conducted by Hayakawa et al. confirming the presence of CTX-M-*E.coli* in 85% of ESBL-producing *E.coli* strains. CTX-M-ESBL strains were associated with the presence of *bla* (beta-lactamase) gene (24).

Worldwide, emergence of the ST131 *E.coli* producing CTX-M is mainly because of the two reasons (i) vertical transmission of the ST131 clone containing CTX-M and (ii) horizontal transfer of plasmids or genes carrying $bla_{CTX\text{-}M\text{-}15}$ alleles (25). Prevalence of CTX-M-15 production is found more to be associated with ST131, followed by CTX-M-14 (13).

Recombinational events occur at molecular levels resulting in well-established plasmids and facilitating the spread of $bla_{CTX\text{-}M\text{-}15}$. The mechanism of these events includes the insertion of sequences of $bla_{CTX\text{-}M\text{-}15}$ genes and the use of expanded spectrum cephalosporins and fluoroquinolones. These events result in the acquisition of resistance to multiple antimicrobials by various mechanisms, particularly to β-lactams, fluoroquinolones, amino-glycosides and trimethoprim.

In addition to the acquisition of $bla_{CTX\text{-}M\text{-}15,}$ multiple point mutations have been observed to be the source of heightened resistance in ST131 ESBL. Quinolone resistance is due to the gyrA and parC genes that encode the target for quinolones. Studies of whole-genome SNP-based phylogenetics have demonstrated that ST131 isolates that are fluoroquinolone resistant are from a discrete subclone H30-R, which is further a part of the H30-subclone group (defined by the fimH-30 allele). Within H30-R is an additional subclone H30-Rx, which is highly associated with the presence of CTX-M-15. The biological mechanism for the spread of H30-R and H30-Rx compared to other ST131 subclones is uncertain. The apparent cause may be the increased anti-microbial resistance, which results in a more difficult to treat infection that results in increased proliferation.

Correspondingly, the virulence and resistance traits of ST131 are debated. An analysis done by Gibreel suggested that not only ST131 but also ST127 have high virulence potential(26).

Colpan's study focused on the relationship between virulence and anti-microbial resistance with ST131 clone. They divided *E.coli* isolates into three different antimicrobial resistance groups (1) fluoroquinolones resistant *E.coli* (FQREC), (2) ESBL *E.coli* and (3) fluoroquinolones susceptible *E.coli* (FQSEC). Colpan and his workers found out high prevalence of ST131 among FQREC and ESBL *E.coli* groups. The virulence profile of ST131 was almost same across the three groups. On the other side, non-ST131 had shown less virulence, as it was less

prominent in FQREC and ESBL *E.coli* than FQSEC groups. The study stated the strong link between ST131 and enhanced virulence (27).

Highly virulent potential plus anti-microbial resistance of ST131 which leads to treatment failure, combined with the use of broad spectrum of antimicrobial agents in infected patients would be the one of the possible explanations for the rapid spread of ST131 among vulnerable patients.

Another study by Kudinha et al. stated the enhance virulence of ST131 in men with pyelonephritis or cystitis. They studied the distribution of ST131 *E.coli* among 101 pyelonephritis, 153 cystitis and 135 healthy-control isolates. They found a high prevalence of ST131 among pyelonephritis isolates and cystitis, indicating a possible link between ST131 and serious infection (28).

In contrast, Lopez-Cerero et al. did not find any difference in the rates of bacteremia; invasive sepsis and mortality at 30 days when ESBL *E.coli* ST131 isolates were compared against ESBL *E.coli* non- ST131 isolates. It is possible these results might be due to a small sample size (29).

Few studies have investigated the clinical outcomes of ESBL *E.coli* ST131 infections and studied the comparison with ESBL *E.coli* non-ST131 infections. Chung et al. conducted research in patients with bacteremia caused by ESBL *E.coli* ST131 and ESBL *E.coli* non-ST131 isolates. They included an analysis of 28-day mortality, which included cancer, shock and community onset of bacteremia as independent factors on multivariate analysis, although, and ST131 was not included as independent predictor of mortality. They found no difference between

the rate of occurrence of bacteremia among ESBL *E.coli* ST131 and ESBL *E.coli* non-ST131 isolates (30).

Banerjee et al. found out that the ST131 clone is not associated with treatment failure or mortality at 6 months. This study included ST131 as an independent predictor on multivariate analysis (31).

The increasing prevalence of ESBL producing *E.coli* showing resistance against fluoroquinolones and cephalosporins and the association of ESBL producing *E.coli* with particular clone ST131 has been reported worldwide (32). Also ST131 is a reason for wide range of *E.coli* infections, especially it's the most common source of urinary tract infections(13). There have been fewer studies reported that compare the clinical outcomes, virulence and antimicrobial resistance occurring due to ESBL producing *E.coli* ST131 infections and ESBL producing *E.coli* non-ST131 infections. In this study, we have compared the characteristics and clinical outcomes of patients with ESBL *E.coli* ST131 infections and patients with ESBL *E.coli* non-ST131 infections using rapid molecular detection of ST131. We hypothesized that the ST131 is the major contributing factor in treatment failures, leading to severe clinical outcomes, in patients with ESBL producing *E.coli* infections. In addition, to gain more insight about the ST131 clone, we analyzed the association of CTX-M-15 with ESBL producing *E.coli* ST131 and ESBL producing *E.coli* non-ST131.

CHAPTER 2 "MATERIAL AND METHODS"

2.1 SPECIMEN COLLECTION

ESBL *E.coli* isolates were collected from a previously conducted retrospective study of ESBL-producing *E.coli*. This retrospective study compared CTX-M *E.coli* isolates with non-CTX-M *E.coli* isolatesand included all patients with an ESBL *E. coli* detected by the clinical laboratory during the study period (February 2010 through July 2011), while receiving care as an inpatient or in the Detroit Medical center emergency room. A total of 377 isolates were obtained (24).

Adult inpatients were included in the study. All medical records were reviewed to abstract the patient's demographics, medical history, clinical course, illness severity and other clinical variables.

2.2. MOLECULAR CHARCTERIZATION

Isolate DNA was extracted (Wizard® Genomic, DNA purification kit, Promega). ST131 characterization was tested by using a two-target multiplex PCR amplification assay (*trpA* and *pabB*). The *pabB* gene is used in the Institute Pasteur MLST (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html) and is one of the genes used to identify the isolates belonging to the O25b-ST131 clone. The primer O25pabB.F (5′-TCCAGCAGGTGCTGGATCGT-3′) and O25pabB.R (5′-GCGAAATTTTTCGCCGTACTGT-3′) were used to amplify a 347 base-pair fragment of the *pabB* gene. The primer sequences were obtained from the MLST database as reported in Clermont *et al* (33). This two-target multiplex PCR amplification is a rapid detection method for the presence of the O25b-ST131 clone. The mentioned

assay included a positive PCR control for the successful amplification of the specific primer- *pabB* allele. The positive control was run so that negative results (i.e. no amplification) is known to not be because of quality concerns including poor DNA quality or failure of the PCR reaction. The control PCR targeted a 427 base-pair fragment of the *trpA* gene, which is another gene included in the Institute Pasteur MLST scheme. This target was amplified with primers *trpA.F* (5′-GCTACGAATCTCTGTTTGCC-3′) and trpA.R (5′-GCAACGCGGCCTGGCGGAAG-3′)(33).

2.3 THERMO-CYCLE CONDITIONS

PCR was carried out in a 50 µL volume containing 25 µL of 20x Master Mix, 12 µL of molecular water, 5 µL of the *pabB* primers and 3 µL of the *trpA* primers, and 5 µl of purified DNA (Wizard® Genomic, DNA purification kit, Promega). PCR tubes were used for the final mixture and following thermo-cycle conditions were used: denaturation 10 min at 95°C, 30 cycles of 30 s at 95°C, 1 min at 60°C and 1 min at 72°C and a final extension step of 5 min at 72°C with a final hold at 4°C. PCR products were loaded on 2% agarose gel with SYBR® Safe DNA gel stain and 1x TBE solution. After electrophoresis, gels were photographed under Blue light. Results with both the trpA and pabB bands identified the O25b-ST131 clone (figure 2).

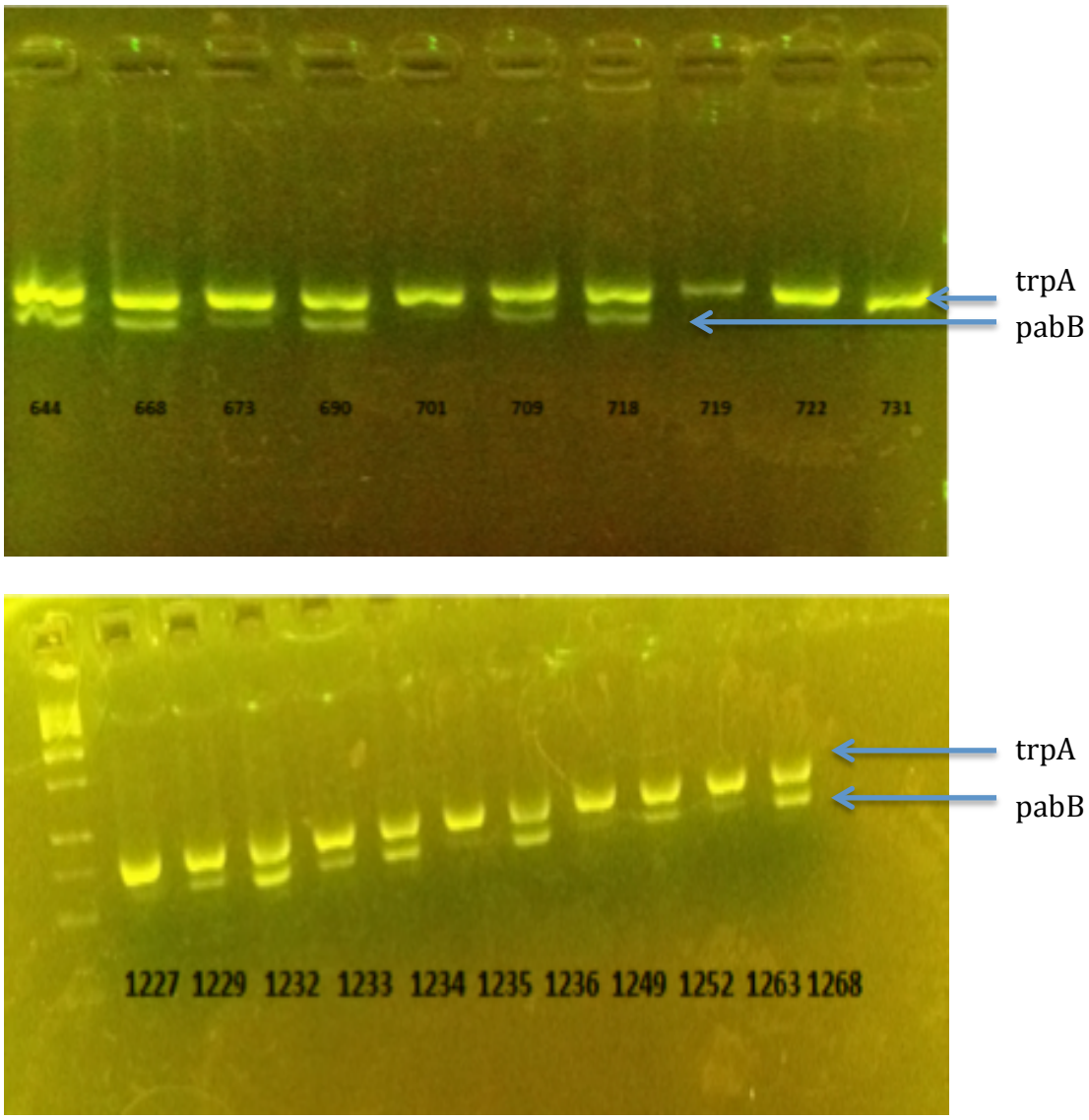

FIGURE 1. GEL PICTURES: PCR detection of *Escherichia coli* O25b-ST131 clone The presence of both the bands trpA and pabB indicates the ST131 existence.

In figure 1, the upper band is *trpA* and the lower band is *pabB*. Presence of both the bands confirms the O25b-ST131 clone. The numbers labeled in the pictures

are the isolate numbers. All were ST131 except 701,719, 722, 731, 1227, 1235 and 1249.

2.4. VALIDATION OF THE RAPID DETECTION ASSAY

In this study we observed ST131 is highly prominent among the ESBL *E.coli* isolates. About 83.7% isolates were ST131. To confirm whether our results were valid, we performed validation of a selection of samples using MLST.

For the validation and confirmation of the rapid PCR assay, eight positive samples and 4 negative samples were randomly selected and MLST assay was performed on them. MLST assay is the standard assay, and includes testing of all the seven house-keeping genes that are adk, fumC, icd, gyrB, mdh, purA, and recA (http://mlst.ucc.ie) (30). Our results were accurate, when both MLST and rapid PCR assay results were compared. Additionally all samples testing negative by the ST131 rapid PCR method were tested a second time for confirmation.

2.5. STATISTICAL ANALYSIS

The characteristics of patients with ST131 ESBL *E.coli* were compared to patients with non- ST131 ESBL *E.coli* using SPSS statistical software. Odds ratios and 95% confidence interval were generated for the comparison. P-values were calculated using chi-squared tests (p-values < 0.05 considered significant).

Two logistic regression models were performed using SPSS to compare clinical outcomes between ST131 and nonST131 groups as follows: Model 1: Dependent variable: Died in 3 months; Independent variable: ST131; and Model 2:

Dependent variable: Died in 3 months; Independent variables: ST131 and Receipt of effective therapy < 72 hours.

CHAPTER 3 "RESULTS"

3.1 PATIENT/ISOLATE DISCRIPTION

377 patients were considered for analysis and complete clinical and molecular data were available for 369 (97.8%). 160 (43.3%) were male. Age was categorized into three groups: 18-30, 40- 64 and patients who were 65 years of age or older (Table 1), with the most frequent age group overall being those who were 65 years of age or older. In most of the cases, urine specimens were recovered (281 isolates; 74.5%), the remaining specimens were from blood (27 isolates), sputum (24 isolates), wound (14 isolates) and others (31 isolates).

3.2 PHYLOGENETIC AND SEQUENCE TYPE DISTRIBUTION

The objective of this cohort study was based on the detection of single ST group, i.e. ST131. From 369 isolates (of 377 total isolates in which 8 were missing), 309 were determined to be ST131 (83.7%). The distribution of ST131 within the group varied significantly among patient's age, and ST131 was highly prominent among the patients who were 65 years of age or older (60.8%)(Table 1), although there was no significant difference when compared with non-ST131 (61.7%) (p-value=0.33). However we found a significant difference when 40-64 age group was compared between clonal groups, the percentage with ST131 in the 40-64 age range was 32% and with non-ST131 were 21.7% with p-value<0.01 (Table 1). ST131 distribution also did not vary significantly by gender, males comprised 44.3% of patients with ST131 isolates and 38.3% of those with non-ST131 isolates (p-value=0.39) (Table 1).

ST131 was present among patients with clinical symptoms of pneumonia (29.4%) and UTI (31.7%) but there were no significant difference in distributions which compared to non-ST131, with p-value=0.66 and p-value= 0.62 respectively (Table 1).

Out of 377 ESBL-producing *E. coli* isolates, the presence of CTX-M was detected in 325 isolates (86.2%). Of 325 CTX-M isolates, 283 isolates (76.7%) specifically had CTX-M-15 detected. Among ST131 group 245 isolates (79.3%) were CTX-M-15 (p-value < 0.01, compared to non-ST131). Whereas 51 isolates total of 325 CTX-M isolates (13.8%) had CTX-M-14 detected and among ST131, 41 isolates (13.3%) were of CTX-M-14 (p-value = 0.49) (Table 1).

ST131 distribution was found to vary by severity of infection. 14.5% of isolates of ST131 were detected with multi-organ failure and 51.4% of isolates of ST131 were found to be associated with severe sepsis. However there was no significant difference among ST131 and non-ST131 groups (p-value=0.66 and 0.62 respectively).

Clinical outcomes including in-hospital mortality, three month mortality and in-hospital surgery were compared between ST131 and non-ST131 groups and they did not vary significantly (Table 2). There was a significant difference in additional hospitalization between two groups with p-value=0.04 (Table 2). Hours to effective therapy were compared and were not found to be significantly different (Table 3).

TABLE 1

CLINICAL CHARACTERISTICS OF PATIENTS:

Characteristics	All (n=366)	ST131 (n=306)	non-ST131 (n=60)	OR (95% CI)	p-value
	Number of Isolates (% column)				
AGE (yrs.)					
18-39	31 (8.4%)	21 (6.8%)	10 (16.7%)	0.365 (0.162-0.820)	reference
40-64	112 (30.4%)	99 (32.0%)	13 (21.7%)	1.704 (0.881-3.294)	p-value< 0.01
≥65	225 (61.0%)	188 (60.8%)	37 (61.7%)	0.965 (0.547-1.705)	p-value= .33
GENDER					
M	160 (43.3%)	137 (44.3%)	23 (38.3%)	1.281 (0.727-2.258)	p-value= .39
PHYLOGENTIC DISTRIBUTION:					
CTX-M-15	283 (76.7%)	245 (79.3%)	38 (63.3%)	2.216 (1.225-4.009)	p-value < 0.01
CTX-M-14	51 (13.8%)	41 (13.3%)	10 (16.7%)	0.765 (0.360-1.626)	p-value= .49
SEPSIS LEVEL:					
MULTIORGAN - FAILURE	55 (14.9%)	45 (14.5%)	10 (16.6%)	0.852 (0.403-1.802)	p-value= .66
SEVERE SEPSIS	192 (52.0%)	159 (51.4%)	33 (55.0%)	0.867 (0.498-1.511)	p-value= .62
ICU TRANSFER	53 (14.3%)	48 (15.5%)	5 (8.3%)	2.023 (0.770-5.315)	p-value= .15
CLINICAL SYMPTOMS:					
PNEUMONIA	107 (28.9%)	91 (29.4%)	16 (26.6%)	1.147 (0.616-2.139)	p-value= .66
URINARY TRACT INFECTIO N	119 (32.2%)	98 (31.7%)	21 (35.0%)	0.863 (0.482-1.543)	p-value= .62
RECENT HOSPITALIZATION	205 (55.5%)	169 (54.6%)	36 (60.0%)	0.805 (0.458-1.413)	p-value= .45

TABLE 2

CLINICAL OUTCOMES FEATURING ACCORDING TO ST131 AND OTHER ISOLATES OF *ESCHERICHIA COLI*.

Characteristics	All (n= 372)	ST131 (n=309)	non-ST131 (n=60)	OR (95%CI)	p-value
Died in hospital	20 (5.4%)	18 (5.8%)	2 (3.3%)	1.794 (.415-.7.94)	p = .44
Died in three months	21 (5.7%)	17 (5.5%)	4 (6.7%)	0.815 (.264-2.51)	p = .72
Additional hospitalization	189 (51.2%)	151 (48.9%)	38 (63.3%)	0.553 (0.313-.979)	p = .04
Invasive surgery	137 (37.1%)	112 (36.2%)	25 (4.7%)	0.796 (.453-1.40)	p = .43
Effective therapy	223a (62.8%) na=Patients (n=355)	190b (64.4%) nb=ST131 (n=295)	33c (58.0%) nc=non-ST131 (n=57)	1.316 (.739-2.24)	p = .35

TABLE 3

HOURS TO EFFECTIVE THERAPY WHEN EXPOSED TO ST131 AND OTHER ISOLATES OF *ESCHERICHIA COLI*.

Total Patients (n=223)[a]	ST131 (n= 187)	non-ST131 (n= 33)	p-value
Effective therapy < 72 hours			
147 (65.9%)	122 (65.2%)	25 (75.8%)	p-value = 0.23
Effective therapy ≥ 72 hours			
73 (32.7%)	65 (34.7%)	8 (24.2%)	

n[a]= The total number of patients are those who underwent the effective therapy.

3.3 RESISTANCE DISTRIBUTION

The resistance profiles of the isolates against antimicrobial agents were collected as part of the previous study (23). The study collected the anti-microbial susceptibility and MICs results of ESBL-producing *E.coli* from Detroit Medical Center from February 2010 through July 2011. The study provided the MICs breakpoints for non-susceptibility of antibiotics. Susceptibility testing of *E.coli* was performed using an automated-broth microdilution system according to the Clinical and Laboratory Standards Institute criteria and the positive ESBL tests were confirmed by disc diffusion test (23). Antimicrobial resistance profiles of ST131 isolates were compared with antimicrobial resistance profiles of non-ST131 isolates (Table 4). The ESBL *E.coli* isolates showed high rates of resistance to most antibiotics especially to β- lactams and fluoroquinolones. A significant difference was found in the prevalence of fluoroquinolone resistance in ST131 isolates compared to non-ST131 isolates. We compared two fluoroquinolones ciprofloxacin and levofloxacin, and the p-value was same in both the cases (p=0.03)(Table 4). Also, we observed a high proportion of resistance against cefepime by ST131 isolates (82.2%), but it was not significantly different from the ST131 group.

TABLE 4

ANTIMICROBIAL RESISTANCE AMONG ST131 ISOLATES OF *ESCHERICHIA COLI*

Number of Isolates (% column)

Antimicrobial	All (n=366)	ST131 (n=306)	non-ST131 (n=60)	OR (95% CI)	p-value
Ampicillin	362 (99.0%)	301 (98.4%)	58 (96.7%)	2.07 (.39-10.9)	0.38
Ciprofloxacin	329 (89.9%)	279 (91.2%)	49 (81.7%)	2.32 (1.08-4.98)	0.03
Levofloxacin	329 (89.9%)	279 (91.2%)	49 (81.7%)	2.32 (1.08-4.98)	0.03
Cefepime	300 (82.0%)	254 (82.2%)	44 (73.3%)	1.78 (0.93-3.39)	0.08
Tetracyclines	217 (59.3%)	181 (59.1%)	35 (58.3%)	1.03 (0.59-1.81)	0.90
Gentamycin	168 (46.0%)	142 (46.4%)	23 (38.3%)	1.39 (0.79-2.45)	0.25
Tobramycin	205 (56.0%)	175 (57.2%)	30 (50.0%)	1.33 (0.77-2.33)	0.30
Piperacillin/tazobactam (Zosyn)	36 (9.83%)	28 (9.15%)	8 (13.3%)	0.66 (0.28-1.16)	0.31

3.4 LOGISTIC REGRESSION ANALYSIS

Regression analysis was performed using SPSS software.

MODEL 1.

TABLE 5

MULTIVARIATE ANALYSIS PREDICTING LIKELIHOOD OF PATIENTS DIED WITHIN THREE MONTHS AFTER BEING EXPOSED TO ST131

Variable	Odd ratio (95%confidence interval)	p-value
ST131	1.136 (0.318-4.056)	0.845

The odds ratio for the association between ST131 *E.coli* infection, compared to non-ST131 infection, and death within three months is 1.136. However, the confidence interval (95% C.I.: 0.318, 4.059) and the p-value (p=0.845) indicate that this association is not statistically significant.

MODEL 2.

TABLE 6

MULTIVARIATE ANALYSIS PREDICTING LIKELIHOOD OF PATIENTS DIED WITHIN THREE MONTHS AFTER BEING EXPOSED TO ST131 AND RECEIPT OF EFFECTIVE THERAPY LESS THAN 72HRS

Variable	Odd ratio (95%confidence interval)	p-value
ST131	**2.635 (0. .325-21.352)**	**0.364**
Receipt of effective therapy Less than 72hrs	**0.312 (0.067-1.464)**	**0.140**

In this model too, the magnitude of the association with ST131 is controlled for the effect of timing of effective therapy. Individuals with ST131 are at high risk (odd ratio is 2.635) of death within three months of infection, however, similar to other models, this model is not statistically significant with p-value > 0.05.

CHAPTER 4 "DISCUSSION AND CONCLUSION"

4.1. DISCUSSION

In this study, we characterized the ESBL *E.coli* ST131 isolates that were obtained from the Detroit Medical Center health care system from February 2010 through July 2011. The prevalence of ST131 varied according to the patient age, phylogenetic distribution and fluoroquinolones resistance. In age group 40-64, there was a significant difference between the groups with p-value<0.001, individuals in this age group were more common in the ST131 group (32%) than in the non-ST131 group (21.7%) (Table1). We observed no statistical difference in prevalence of ST131 by gender.

CTX-M-15 was observed in 76.7% of ESBL *E.coli* isolates, and observed in 79.3% of ST131 isolates. Our data corresponds to reports from others that ST131 is largely responsible for the spread of CTX-M-15 resistance elements globally (28). There is a significant difference in occurrence of CTX-M-15 between ST131 (79.3%) and non-ST131 (63.3%) with p-value<0.01 (Table 1). CTX-M-14 was also reported with 13.8% of total isolates, but it was not statistically significant.

Urinary tract infections (32.2%) and pneumonia (28.9%) were observed most frequently in our study population of patients with ESBL *E.coli* infections. In this study, ESBL *E.coli* ST131 identification was mostly confined to urine, reflecting the distribution of the overall population.

We also compared hours to effective therapy between the patients infected with ST131 and patients with non- ST131 (Table 3). There was no significant

difference, although effective therapy given within 72 hours was more common in case with patients with non-ST131 (75.8%) and effective therapy given after 72 hours was present more often in patients with ST131 (34.7%) compared to non-ST131.

ST131 showed high resistance against fluoroquinolones. Risk of existing resistance against ciprofloxacin was more prominent among patients with ST131 (91.2%) as compared to patients with non-ST131 (81.7%) with p-value=0.03, shown in Table 4. As with ciprofloxacin, levofloxacin resistance was more seen among patients with ST131 (91.2%) when compared to patients with non-ST131 (81.7%) with p-value=0.03 (Table 4). We also observed a high prevalence of resistance against β-lactams and carbapenems, but the comparison was not statistically significant.

The strength of this study was the larger sample size and the availability of ESBL *E.coli* isolates, which were obtained from a previously conducted retrospective study of sequential clinical isolates. For characterization, we used two-target multiplex PCR amplification (trpA and pabB), which is a preferred technique to standard MLST as its results are more efficient and large studies are easily conducted by this method. The two-target multiplex PCR amplification assay is based on detecting two single nucleotide polymorphisms (SNPs), namely thymine-144 and adenine-450, in *pabB*. This combination is unique to ST131 and the occurrence of both of these *pabB* SNPs implies that an isolate belongs to ST131 (34).

The primary study limitation was that we did not confirm the two-target multiplex PCR amplification (trpA and pabB) with standard MLST for each isolate.
However, we choose a selection of isolates from two groups i.e. ST131 and non-ST131, and did the standard MLST PCR for confirmation.

4.2 CLINICAL OUTCOMES

We performed a multivariate analysis comparing patients with ESBL *E.coli* ST131 and ESBL *E.coli* non-ST131 isolates. We investigated independent predictors of three-month-mortality, including ST131 and effective therapy within 72 hours. This is an important confounder, as this investigation has not been performed in previous studies. The study concluded that there is high mortality among patients with ST131, as out of 20 patients who died in hospital, 18 patients were with ST131 and only 2 were with non-ST131. However, there was no statistical significance when two groups were compared.

4.3 CONCLUSION

The global emergence of ST131 is one of the major reasons for the spread of ESBL *E.coli* infections. ST131 can be considered the driving factor for the spread of CTX-M-15. There is an increased rate of resistance against antibacterial agents i.e. fluoroquinolones and cephalosporins due to over exposure to antibiotics. With continued antibiotic exposure, it is possible that the ST131 clone can develop resistance against fosfomycin and pivmecillinam, which are empiric treatments for ESBL *E.coli* infections. Though we did not find any significant clinical difference between the infections caused by ESBL *E.coli* ST131 and infections caused by ESBL

E.coli non-ST131, despite controlling for time to effective therapy, we found a remarkably high prevalence of ST131 among patients treated for *E. coli* infection. In summary, we did not find any evidence that ST131 is more virulent than non-ST131 as there was no significant difference in clinical outcomes between the two groups.

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ABSTRACT

HIGH PREVALENCE OF ST131 AMONG EXTENDED SPECTRUM B-LACTAMASE PRODUCING *E. COLI* AMONG INPATIENTS IN THE METROPOLITAN DETROIT AREA

by

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August 2015

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Objectives: *E.coli* ST131 multi-locus sequence type (MLST) has been associated with extended spectrum β-lactamase (ESBL) production, conferring antimicrobial resistance, with increased virulence and with healthcare-associated infection. The high prevalence of multidrug antimicrobial resistance in ESBL-producing ST131 *E.coli* infections creates unique challenges in the studying patient outcomes and analysis are required to study ST131 *E.coli* amongst a large population of ESBL-producers. Our objective was to determine the prevalence of ST131-type *E. coli* among previously characterized ESBL-producing isolates obtained from the metropolitan Detroit area and to conduct an epidemiologic analysis of risk factors and illness severity.

Methods: 377 ESBL-*E.coli* isolates were obtained from a previously conducted retrospective study of sequential clinical isolates obtained from the

Detroit Medical Center health care system from February 2010 through July 2011. Isolate DNA was extracted and tested for ST131 characterization using a two-target multiplex PCR amplification (*trpA* and *pabB*). Patient demographics, medical history, clinical course, and illness severity were collected retrospectively from medical records, and *E. coli* susceptibility was obtained from the clinical laboratory. Predictors and outcomes of infection due to ST131 ESBL *E. coli* were compared to non-ST131 ESBL *E. coli*.

Results: 81.9% (n=309) of 377 tested isolates were positive for the ST131-specific *pabB* allele. Patients with ST131 versus non-ST131 EBSL *E.coli* were comparable with respect to age, race, and intensive care unit admission. 76.7% (n=283) of the ST131 isolates had CTX-M-15 and 13.8% (n=51) had CTX-M-14 present. However, the presence of CTX-M was not limited to the ST131 group, 63.3% (n = 38) of non-ST131 isolates had CTX-M-15 and 16.7 (n=10) had CTX-M-14 present.

Conclusion: we found out the high prevalence of ST131 among patients with *E.coli* infections. However, we detect no significant clinical difference between the infections caused by ST131 *E.coli* and non-ST131 *E.coli*.

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