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Prevalence of *Escherichia Coli* sequence type 131 (ST131) among extra-intestinal clinical isolates in different phylogenetic groups

Al-hetar Khadega Yahyah Abdullah and Nanjaiah Lakshmidevi

Department of Studies in Microbiology, University of Mysore, Mysore, India
Corresponding Email: K_yah2007@yahoo.com

ABSTRACT

Escherichia coli sequence type O25b-ST131 has emerged in recent years around the world in combination with CTX-M-15 among extra-intestinal infections and it has caused considerable morbidity and mortality. Management of infections can be done by controlling the source of infections or contamination. In India there are not many studies on O25b-ST131 clone, This study was done to investigate ST131's prevalence in different phylogenetic groups among isolates from different extra-intestinal infections. Hundred E. coli isolates were isolated from different clinical samples including urine, blood, sputum and exudates from Krishna Rajendra (K.R) Hospital, Mysore. Polymerase chain reaction (PCR) method was used to determine the Phylogenetic groups and O25b-ST131 clone. Out of 100 E. coli strains 55%, 30%, 8% and 7% were isolated from urine, exudates, sputum, and blood respectively. Phylogenetically, B2 group was the dominant group (34%) followed by D, B1 and A groups (28, 26, and12) respectively, 87% E. coli samples belonged to O25b-ST131 clone which were distributed among groups; B2, D, B1 and (32, 25, 22 and 8 isolates respectively). Diversity of Phylogenetic groups among O25b-ST131 E. coli strains may refer to the source of infections. Further studies are needed to highlight the source of infection which may help to control the dissemination of E. coli clone.

Keywords: *E. coli*, ExPEC, O25b-ST131 clone, PCR, Phylogenetic groups

INTRODUCTION

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) strains are a clinically significant group of pathogens that cause a variety of clinical syndromes that include urinary tract infections, nosocomial pneumonia, neonatal meningitis, and sepsis [1]. Phylogenetic analysis of *E. coli* species have shown that the majority of strains belong to four phylogenetic groups: A, B1, B2, and D related to the genetic markers *chuA*, *yjaA* and the DNA fragment *TspE4.C2* [2]. To increase the discrimination power of *E. coli* population analyses, subgroups A0, A1, B1, B22, B23, D1 and D2 are determined by the combination of the genetic markers as follows: subgroup A0 (group A), *chu*A-, *yja*A-, Tspe4.C2-; subgroup A1 (group A), *chuA*-, *yja*A+ Tspe4.C2-; group B1, *chu*A-, *yja*A-, Tspe4.C2+ subgroup B22 (group B2), *chu*A+, *yja*A+, Tspe4.C2-; subgroup B23 (group B2), *chu*A+, *yja*A+ Tspe4.C2+ subgroup D1 (group D), *chu*A+, *yja*A-, Tspe4.C2-; and subgroup D2 (groupD), *chu*A+, *yj*A-, Tspe4.C2 [3,4]. Commensal strains fall into groups A and B1, whereas extra-intestinal *E. coli* (ExPEC) mainly belong to group B2. However, Diarrhoeagenic strains fall into groups A, B1, and D [5,6]. Extra-intestinal infections related to *E. coli* cause considerable morbidity, mortality, and increased health care costs. Johnson et al. [7] reported that *E. coli* strains that were resistant to antibiotics shifted towards phylogenetic groups A and D, and moved away from group B2. Previous studies have also shown diversity in phylogenetic groups for ExPEC based on the host; most of the ExPEC isolates from animal origin belongs to commensal groups A and B1 while human origin isolates belong to pathogenic groups B2 and D [8]. A new clone of *E. coli* named ST131 that is belonging to phylogenetic group B2 and associating with serogroup O25b was identified in nine countries including Canada, India, Kuwait, France, Switzerland, Portugal, Lebanon, Korea, and Spain [9, 10]. This clone is the dominant strain among Extra-intestinal pathogenic *E. coli* and has been reported to cause a wide range of infections including meningitis, osteomyelitis, myositis, epididymio-orchitis, and peritonitis [11-14]. In addition it is most commonly associated with urinary tract infections in the United States [11-13]. Clarification of the evolutionary origins of ExPEC conceivably could

facilitate the development of novel measures to block their continued emergence [15]. In this study, phylogenetic groups and sequence type O25b-ST131 of *E. coli* isolates that have been isolated from different extra-intestinal infections have been investigated.

MATERIALS AND METHODS

Study design: Epidemiological

Bacterial isolates

A total of 100 of *E. coli* clinical isolates were collected from K. R. hospital, Mysore, India. All isolates were isolated from different extra-intestinal specimens including urine, blood, sputum and exudates over a period of 10 months (June 2013 to March 2014). The inclusion criteria are: the gender (both of male and female are used) and the age of patients (adults (16-55yrs), elderly (> 56 yrs) and children (0-15 yrs)). However, the exclusion criteria were the type of infection. All isolates were identified as *E. coli* by inoculating them on MacConky Agar (M.A.) and Eosin Methylen Blue (E.M.B.) Agar and by using biochemical identification kit (HiE. coli Identification Kit, HiMedia Laboratories Pvt. Ltd, Mumbai, India).

Phylogenetic analysis

Template DNA was prepared by boiling method as described by Ruppe et al. [16]. Based on Clermont dichotomous tree, the amplification of *chuA*, *yjaA* and *TspE4.C2* genes were carried out using the primers and PCR conditions as mentioned in the previous report [2]. The amplified products were analysed in an agarose gel with a 1.2% gel and the amplicon size was measured by using 100 bp DNA Ladder (GeNei^{TM}, Mumbai, India). Electrophoresis was carried out at 70V, 500A for 90 mins.

Determination of O25-ST13 *E. coli* by PCR for the *pabB* gene

PCR detection of the O25b-ST131 clone isolates Primers O25pabBspe.F (5'-TCCAGCAGGTGCTGGATCGT3') and O25pabBspe.R (5'GCGAAATTTTTCGCCGTACTGT3') were used to amplify a 347 bp fragment of the pabB gene. PCR method was performed as mentioned before [17].

Statistical analysis: Chi Square was used to compare between phylogenetic groups; commensal and pathogenic groups.

RESULTS

Hundred *E. coli* isolates were collected from extra-intestinal clinical samples. The demographical data showed that 44%, 30%, and 26% isolates were isolated from adults (16-55yrs), elderly (> 56 yrs) and children (0-15 yrs) from both genders (55% from female and 45% from male).

Out of 100 ExPEC samples, 55% 30%, 8%, and 7% were isolated from urine, exudates, sputum and blood respectively. The highest prevalence of *E. coli* was from females, mostly from urine. The distribution of *E. coli* strains among urine samples is 37 out of 55(67.3%) for females and 18 out of 45 (40%) for males, the differences between them were strongly significant (P<0.006).

Phylogenetic analysis of *E. coli* isolates that were isolated from extra-intestinal infections showed that 35, 28, 26 and 11 isolates belonged to groups B2, D, B1 and A respectively of which group B2 is the predominant (47.9%) among urine samples. However, the most prevalent was for pathogenic groups B2 and D, the difference between pathogenic and commensal groups was significant (P<0.04). B2 and D groups were the most prevalent among urine samples 23 (41.8%) and 16 (29.1%) isolates respectively, while B1 and D were the most prevalent among exudates samples 10 (33.3) and 8 % (26.7%) isolates respectively. However, among blood samples, group B2 was the most prevalent with 3 (42.8%) isolates. The difference is not significant for sputum and blood samples (Table 1& Fig.1).

Prevalence of O25b-ST131 strain among ExPEC isolates

Of 100 ExPEC isolates, 87 isolates were identified as the O25b-ST131 clone. Presence of ST131 was higher among B2, D, and B1groups (32, 26, and 22 respectively) but the differences in the distribution of ST131 clone among different phylogenetic groups were not significant (P<0.07) which refer to the ability of ST131 clone to transfer among commensal groups. The distribution of ST131 strains among phylogenetic groups was in different proportions; 7 (8.04%) for group A, 24 (25.28%) for group B1, 32 (36.78%) for group B2, and 26 (29.88%) for group D.

DISCUSSION

Extra-intestinal pathogenic *E. coli* (ExPEC) has emerged around the world in recent years in combination with antibiotic resistance among different infections. Out of 100 ExPEC samples, 55% 30%, 8%, and 7% are isolated from urine, exudates, sputum and blood respectively. The highest prevalence of *E. coli* was from female, mostly from urine. Compatible with other studies, 57% and 42% of *E. coli* isolates were isolated from urine and exudates respectively [18], while the prevalence of *E. coli* was low; 22% from urine, and 6% from exudates [19]. The distribution of *E. coli* strains among urine samples is 37 out of 55(67.3%) for females and 18 out of 45 (40%) for males, the differences between them was strongly significant ($P<0.006$). This result is similar to another study which reported that the distribution of *E. coli* isolates was 70% for female [20]. This finding may refer to the shorter urethra of females which may be easily invaded by commensal *E. coli* that is endemic in the intestinal tract after acquisition of virulence factors.

Phylogenetically, Extra-intestinal pathogenic strains usually belong to phylogroups B2 and D, while the commensal strains belong to groups B1 and A, the intestinal pathogenic strains belong to groups A, B1 and D [4]. Groups B2 and D were the predominant groups among UTI infections [21]. In the current study, phylogenetic analysis of *E. coli* isolates that were isolated from extra-intestinal infections showed that 35, 28, 26 and 11 isolates belonged to groups B2, D, B1 and A respectively among which group B2 was the predominant (47.9%) among urine samples. However, the most prevalent was for pathogenic groups (B2 and D), the difference between pathogenic and commensal groups was significant ($P<0.04$). B2 and D groups were the most prevalent among urine samples 23 (41.8%) and 16 (29.1%) isolates respectively, while B1 and D were the most prevalent among exudates samples 10 (33.3%) and 8 (26.7%) isolates respectively. However, among blood samples group B2 was the most prevalent with 3 (42.8%) isolates. The difference was not significant for sputum and blood samples. Presence of phylogenetic groups A and B1 among extra-intestinal infections which have been reported on animals or humans as commensal *E. coli* strains [4] may refer to the source of infection as was mentioned by Johnson et al. [8] who reported that most of the ExPEC isolates from animal origin belongs to commensal groups A and B1 while human origin isolates belong to pathogenic groups B2 and D, or to the resistance of *E. coli* to antibiotics as Johnson et al. [7] reported that, *E. coli* strains that are resistant to antibiotics shifted towards phylogenetic groups A and D and moved away from group B2. This diversity of phylogenetic groups may predict the type of resistance genes especially ESBLs types as previous studies that reported most of CTX-M-15 producing *E. coli* belong to group B2 while CTX-M-9 producing *E. coli* belong to group D [22].

ST131 clone has been reported as a predominant strain that was disseminated in many countries in three continents including Europe, Asia, and North America [9,10]. Of 100 ExPEC isolates, 87 isolates were identified as the O25b-ST131 clone. Presence of ST131 was higher among B2, D, and B1groups (32, 26, and 22 respectively) but the differences in the distribution of ST131 clone among different phylogenetic groups are not significant ($P<0.07$) which refer to the ability of ST131 clone to transfer among commensal groups. Prevalence of ST131 strain has increased worldwide; from 2000-2010 it increased from 0-49% in Canada [23-25], in Nigeria; 35.7% [26], and 64.5% in Saudi Arabia [27]. In India, the first report on ST131 was in 2008 [9,10]. However, the prevalence proportion has increased in different Indian regions 36.66% and 70% of ST131 strains were isolated from urine patients [2, 28]. The current study confirmed the increase of ST131 dissemination; we found 87% of *E. coli* were positive to ST131 clone from different extra-intestinal infections. Phylogenetically, ST131 strains belong to group B2 [24] while Coque and Nicolas-Chanoine [9,10] found two non ST131strains (ST28 and ST354) belonging to group B2 and one ST131 strain belonging to group D. In our study, the distribution of ST131 strains among phylogenetic groups was in different proportions; 7 (8.04%) for group A, 24 (25.28%) for group B1, 32 (36.78%) for group B2, and 26 (29.88%) for group D. This finding may refer to the difference of the source of infections. Diversity of phylogenetic groups of *E. coli* is considered as an index of risk which refers to presence of animal contamination by contaminated food or water and may refers to occurring of antibiotic resistance for most important antibiotics especially beta-lactam antibiotics.

Table 1: **Distribution of the phylogenetic groups on clinical samples and the ST131 strains**

	Urine (55) **No. (%)**	**Exudates(**30) **No. (%)**	**Blood(**7) **No. (%)**	**Sputum(**8) **No. (%)**	**ST131 (87)**
B2	23(41.8)	6 (20)	3(42.8)	2(25)	32(36.8)
D	16(29.1)	8(26.7)	2(28.6)	2(25)	25(28.7)
B1	13(23.6)	10(33.3)	1(14.3)	2(25)	22(25.3)
A	3(5.5)	6(20)	1(14.3)	2(25)	8(9.2)

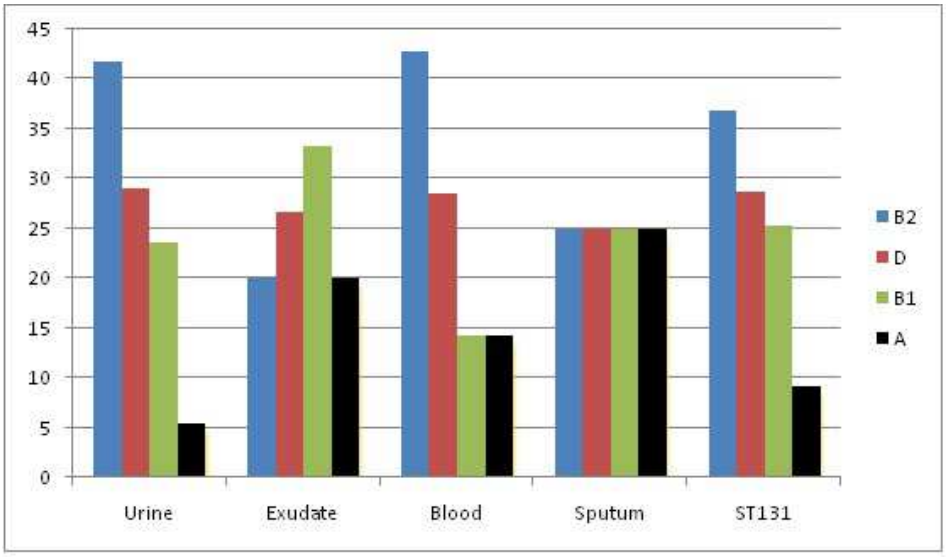

Figure 1: Distribution of the phylogenetic groups on clinical samples and the ST131 strains

CONCLUSION

The presence of ST131 *E. coli* among different phylogenetic groups confirms that there is no relation between ST131 clone and phylogenetic group B2. This finding refers to the ability of ST131 clone to disseminate among commensal groups or sensitive strains. Many studies should be done for further investigations to control the dissemination of ST131 *E. coli* strains.

REFERENCES

[1] Nazareth H, Genagon SA, Russo T. Extraintestinal Pathogenic Escherichia coli Survives within Neutrophils. Infect Immun 2007; 75: 2776–2785.
[2] Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic groups. Appl Environ Microb 2000; 66: 4555-4558.
[3] Escobar-Páramo P, Le Menac'h A, Le Gall T, Amorin C, Gouriou S, Picard B, et al. Identification of forces shaping the commensal Escherichia coli genetic structure by comparing animal and human isolates. Environ Microbiol. 2006; 8(11):1975-84.
[4] Carlos C, Pires M, Stoppe N, Hachich E, Sato M, Gomes T, et al. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 2010; 10:161.
[5] Baldy-Chudzik K, Mackiewicz P, Stosik M. Phylogenetic background, virulence gene profiles, and genomic diversity in commensal Escherichia coli isolated from ten mammal species living in one zoo. Vet microbiol 2008; 131:173-184.
[6] Zhang L, Foxman B, Marrs C. Both Urinary and Rectal Escherichia coli Isolates Are Dominated by Strains of Phylogenetic Group B2. J Clin Microbiol 2002; 40: 3951–3955.
[7] Johnson JR, Kuskowski MA, Owens K, Gajewski A, Winokur PL. Phylogenetic origin and virulence genotype in relation to resistance to flouroquinolones and/or extended-spectrum cephalosporins and cephamycins among Escherichia coli isolates from animals and humans. J Infect Dis 2003;188: 759-768.
[8] Johnson J, Kuskowski MA, Owens K, Clabots C, Singer RS. Virulence genotypes and phylogenetic background of fluoroquinolone-resistant and susceptible Escherichia coli urine isolates from dogs with urinary tract infection.Vet Microbiol 2008; 1-7.
[9] Coque T, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of Clonally Related Escherichia coli Strains Expressing Extended-Spectrum β-Lactamase CTX-M-15. Emerg Infect Dis 2008; 14: 195-200.
[10] Nicolas-Chanoine M, Blanco J, Leflon-Guibout V, Demarty R, Alonso M., Canic M¸ et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J of Antimicrob Chemoth 2008; 61: 273–281.
[11] Johnson J, Johnston B, Clabots C, Kuskowski M, Castanheira M. Escherichia coli Sequence Type ST131 as the Major Cause of Serious Multidrug Resistant E. coli Infections in the United States. Clin Infect Dis 2010; 51:286–294
[12] Vigil K, Johnson J, Johnston B, Kontoyiannis D, Mulanovich V, Raad I, et al. Escherichia coli Pyomyositis: An Emerging Infectious Disease among Patients with Hematologic Malignancies. Clin Infect Dis 2010; 50: 374–80

[13] Assimacopoulos A, Johnston B, Clabots C, Johnson J. Post Prostate Biopsy Infection with Escherichia coli ST131 Leading to Epididymo-Orchitis and Meningitis Caused by Gram-Negative Bacilli. J Clin Microbiol 2012; 50:12: 4157–4159.
[14] Price L., Johnson J., Aziz M., Clabots C., Johnston B., Tchesnokova V., et al. The Epidemic of Extended-Spectrum-_-Lactamase-Producing Escherichia coli ST131 Is Driven by a Single Highly Pathogenic Subclone, H30-Rx. MBio.2013; 4(6):1-10.
[15] Johnson J, Owens K, Clabots C, Weissman S, Cannon S. Phylogenetic relationships among clonal groups of extraintestinal pathogenic Escherichia coli as assessed by multi-locus sequence analysis. Microbes Infect 2006; 8: 1702-1713.
[16] Ruppe E, Hem S, Lath S, Gautier V, Ariey F, Sarthou JL, et al. CTX-M β-lactamases in Escherichia coli from community-acquired urinary tract infectons, Cambodia. Emerg Infect Dis 2009; 15:741-748.
[17] Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, et al. Rapid detection of the O25b-ST131 clone of Escherichia coli encompassing the CTX-M-15-producing strains. J Antimicrob Chemoth 2009, 64: 274-277.
[18] Umadevi S, Kandhakumar G, Joseph NM, Kumar S, Easow JM, Stephen S, et al. Prevalence and antimicrobial susceptibility pattern of ESBLproducing Gram Negative Bacilli. J Clin & Diag Res 2011; 5(2):236-239.
[19] Agrawal P, Ghosh AN, Kumar S, Basu B, Kapila, K. Prevalence Of extended-spectrum β-lactamases among Escherichia coli and klebsiella pneumoni isolates in a tertiary care hospital. Indian J Pathol Microbiol 2008; 51: 139-142.
[20] Jadhav S, Gandham N, Paul A, Misra N, Mani R. Characterization of multiple drug resistant uropathogenic Ecsherichia coli (UPEC) with special reference to B2-O25-ST131-CTX-M-15 India. J Pharm & Biom Scie 2012; 23:1-5.
[21] Cao X, Cavaco L, Lv Y, Li Y, Zheng B, Wang P, et al. Molecular Characterization and Antimicrobial Susceptibility Testing of Escherichia coli Isolates from Patients with Urinary Tract Infections in 20 Chinese Hospitals. J Clin Microbiol 2011; 49: 2496–2501.
[22] Karisik E, Ellington MJ, Livermore DM, Woodford N. Virulence factors in Escherichia coli with CTX-M-15 and other extended spectrum β-lactamases in the UK. J Antimicrob Chemoth 2007; 61: 54-58.
[23] Peirano G, Bij K, Gregson D, Pitout P. Molecular Epidemiology over an 11-Year Period (2000 to 2010) of Extended-Spectrum β-Lactamase- Producing Escherichia coli Causing Bacteremia in a Centralized Canadian Region. J Clin Microbiol 2012; 294–299.
[24] Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of Escherichia coli as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob Agents Ch. 2009; 53:2733–2739.
[25] Pitout J, Gregson D, Campbell L, Laupland K. Molecular Characteristics of Extended-Spectrum-β-Lactamase-Producing Escherichia coli Isolates Causing Bacteremia in the Calgary Health Region from 2000 to 2007: Emergence of Clone ST131 as a Cause of Community-Acquired Infections. Antimicrob Agents Ch 2009; 53: 2846-2851.
[26] Aibinu I, Odugbemi T, Koenig W, Ghebremedhin B. Sequence Typz ST131 and ST10 Complex (ST617) predominant among CTX-M-15-producing Escherichia coli isolates from Nigeria. Clin Microbiol Infec 2011; 18: 49–51.
[27] Al-Agamy M, Shibl A, Hafez M, Al-Ahdal M, Memish Z, Khubnani H. Molecular characteristics of extended-spectrum β-lactamase-producing Escherichia coli in Riyadh:emergence of CTX-M-15-producing E. coli ST131. Ann Clin Microb Anti 2014; 13: 2-7.
[28] Hussain A, Ewers C, Nandanwar N, Guenther S, Jadhav S, Wieler L, et al. Multiresistant Uropathogenic Escherichia coli from a Region in India Where Urinary Tract Infections Are Endemic: Genotypic and Phenotypic Characteristic of Sequence Type 131 Isolates of the CTXM-15 Extended-Spectrum-β-Lactamase-Producing Lineage. Antimicrob Agents Ch. 2012; 56: 6358–6365.