

Virulence Factors of *E. coli* ST131 and Its H30 and H30Rx Subclones Among Extended-Spectrum Beta-Lactamase Producing Isolates

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Abstract: *Escherichia coli* sequence type 131 (ST131) is a pandemic clone causing predominantly community and hospital-onset antimicrobial-resistant infections. Recently, H30-Rx subclone of *E. coli* has been disseminated among ST131 strains in combination with beta lactamase subtype CTX-15 that may play a role in the prevalence of antibiotic resistance among *E. coli* strains that cause extra-intestinal infections. This study has been done to investigate the virulence factors of ST131 with their subclones: H30-Rx and non H30-Rx strains. A hundred *E. coli* isolates which were isolated from different clinical samples were collected from K.R hospital, Mysore. Polymerase chain reaction (PCR) method was used to investigate 19 of the virulence factors among *E. coli* stains. Out of 100 *E. coli* strains, 87 of the isolates belonged to ST131, 24 isolates belonged to H30 subclone, and 22/24 (91.7%) belonged to H30-Rx subclone. ST131 strains harbored high virulence factors more than non ST131 strains and the differences between them were significant for *papC*, *papEF* and *fyuA* genes. All H30-Rx strains were positive for *iutA* and *fimH*, however, 95.5% and 81.8% of H30-Rx strains were positive for *fyuA* and *kpsMIII* respectively and the differences between H30-Rx and non H30-Rx strains were significant for those genes. There are no more reports about H30 and H30-Rx subclones of ST131 *E. coli* in India. Further studies should be done to investigate the prevalence of ST131 in different places in India and to highlight the other virulence factors to control the dissemination of ST131 clone.

Keywords: *E. coli*, ESBL, H30 and H30Rx Subclones, ST131, Virulence Factors

1. Introduction

Escherichia coli sequence type 131 (ST131) is a pandemic clone causing predominantly community and hospital-onset antimicrobial-resistant infection. This clone was the dominant strain among Extra-intestinal pathogenic *E. coli* (ExPEC) and has been reported to have caused a wide range of infections. In addition, it was most commonly associated with urinary tract infections in the United State [1-3]. ST131 strain was detected in combination with beta-lactamases sub type CTX-M-15 that was identified in India in 1999 [4, 5]. Recently, the H30 and H30-Rx subclones of *E. coli* ST131 have been prevalent more extensively than other ST131 variants. The H30 subclone is named because it contains allele 30 of *fimH* (type 1 fimbrial adhesion gene) [6]. Within the H30 subclone, the H30-R subset referred to as the H30

isolates were fluoroquinolone resistant strains while the H30-Rx subset often carries bla_{CTX-M-15} [7, 8]. *E. coli* ST131 clone in association with bla_{CTX-M-15} gene and its H30 and H30-Rx subclones have been prevalent globally as a public health concern. However, no more attention has been given to understand the molecular epidemiology of these strains with their subclones in high-burden countries such as India. Presence of ST131 clone with its subclones H30 and H30-Rx among extra-intestinal infections is considered a risk factor as it increases the morbidity and the mortality by making the infections difficult to treat since they are associated with the resistance to antibiotics especially fluoroquinolone and ESBLs [6, 9]. A study of ST131 clone and its subclones on their harboring of virulence factors (V.Fs) is very important to improve the treatments against them and to control their dissemination. This study has been done to investigate the

virulence factors of ST131 among its subclones; H30-Rx and non H30-Rx.

2. Material and Methods

2.1. Clinical Isolates

One hundred *E. coli* isolates that were collected from K.R hospital, Mysore, India, have been isolated from different clinical samples including urine, exudates, blood and sputum. All isolates have been confirmed by using MacConkey Agar, Eosin Methylene agar (EMB) and biochemical kit (HiE. coli Identification Kit, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and they have been investigated for production of ESBLs and CTX-M subtypes [10].

2.2. Determination of Phylogenetic Groups, ST131 Clone, H30 and H30-Rx Subclones

Investigations of phylogenetic groups and ST131 clone of all isolates have been done using PCR method [11]. However, H30 and H30-Rx subclones have been determined before [12].

2.3. Determination of the Virulence Factors

Template DNA was prepared by boiling method as described by Ruppe et al. [13]. All *E. coli* isolates were screened for the presence of 19 virulence factors; P fimbriae adhesion (*papC*, *papG*, *papA*, *papEF*), S and FIC fimbriae (*sfa/foc*), aerobactin receptor (*iutA*), blood group M adhesion (*bam*), type 1 fimbriae (*fimH*), capsular polysaccharide (*kpsMIII*, *kpsMIII*, *k15*), vaculating autotransporter (*vat*), siderophore receptor (*ireA*), *ibe 10*, α -hemolysin (*hlyA*), O4 LPS synthesis (*rfc*), nonfimbrial adhesin-1 (*nfa*), pathogenicity islands (PAI), and yersiniabactin receptor (*fyuA*) have been detected by PCR using published primers [14]. The PCR reaction was prepared in 25 μ l as a total volume by using 12.5 μ l of Master Mix (GeNei™, Bangalore, India),

0.15 μ l of 100 μ M for each primer (Amnion Biosciences, Bangalore, India) by adding 2 μ l of DNA and completing the volume to 25 μ l by sterilized Distilled water. The PCR conditions are: 95°C for 12 min of activation, 28 cycles of: 94°C for 30 sec of denaturation, 63°C for 30 sec of annealing, 68°C for 3 min of extension, the final extension is 72°C for 10 min and the final hold in 4°C [14]. The virulence score was calculated as the sum of all virulence factors to which an isolate was positive.

Distribution of virulence factors was detected among ST131 clone and its H30-Rx subclone and the differences between H30-Rx and non H30-Rx strains were analysed by using Chi Square test and $P \leq 0.05$ was regarded as cutoff for significance.

3. Results and Discussion

With regard to the previous data, we found that, 87 of 100 (87%) *E. coli* isolates, belonged to ST131 clone, 24/87 (27.6%) strains belonged to H30 subclone, and 22 /24 (91.7%) isolates belonged to H30-Rx subclone [11, 12]. Out of the overall 87 ST131 isolates, 77 (88.5%) isolates carry *CTX-M-15* gene. However, 10 (11.5%) ST131 isolates do not carry the *CTX-M-15* gene [10]. This result is higher than other studies that have found 27.3%, 27%, 25%, and 9.5% of ST131 strains produced *CTX-M-15* in Korea, Olmsted country, Mexico and China respectively [15-18] but it approximate to another studies that have reported that 95%, 89.5% and 71% of ST131 strains produced CTX-M-15 in Spain and Canada, [19-21].

3.1. Virulence Factors of *E. coli* isolates

Nineteen virulence factors have been investigated among 100 *E. coli* isolates from different extra-intestinal clinical samples including urine, exudates, sputum and blood. Only two virulence factors (i.e. *hlyA* or *rfc*) were absent among *E. coli* isolates and the others factors are showed in Figure 1.

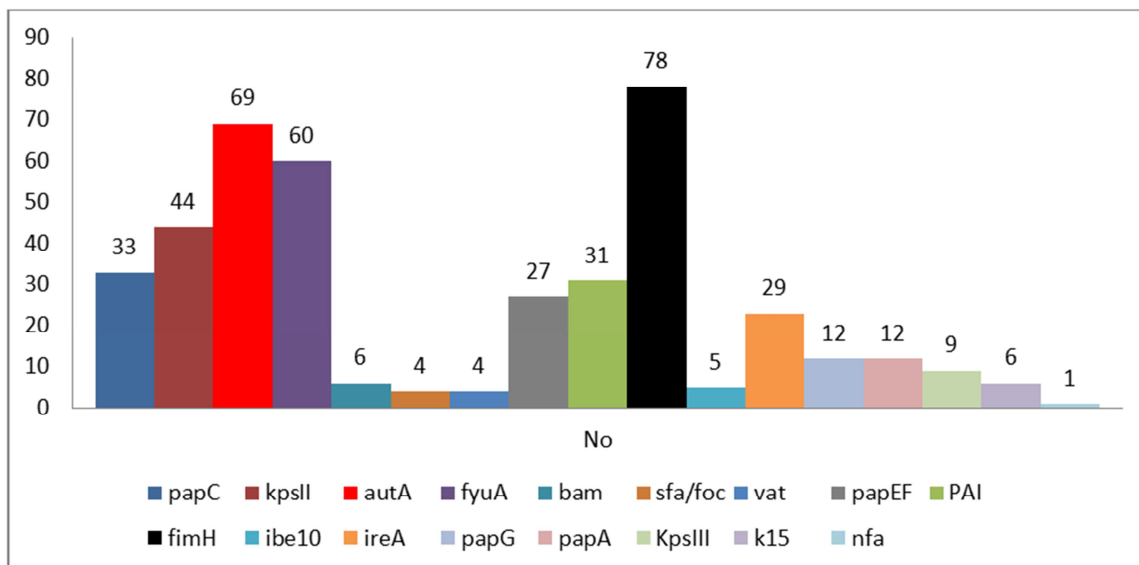


Figure 1. The virulence factors of *E. coli* isolates.

3.2. Distribution of VF among ST131 Clone with Its H30-Rx Subclone Strains and Clinical Samples

Most of virulence factors have been produced from ST131 strains and they are distributed among H30-Rx strains more

than non H30-Rx strains. Factors; *fyuA* and *fimH* are higher among H30-rx strains and the differences for them are significant ($P \leq 0.05$) between H30-Rx strains and non H30-Rx strains as was mentioned in Table 1.

Table 1. Distribution of VF. among ST131 and its H30-Rx subclone Strains and clinical samples.

Category	Gene	exudates	Urine	sputum	Blood	P value for urine samples ver. others	Non ST131 (13)		P of H30-Rx ver. non H30-Rx	
							ST131 isolates (87)	H30-Rx (22) No. (%)		Non-H30Rx (65) No. (%)
Adhesin	<i>papa</i>	2	9	1	0	0.1	0	5 (22.7)	7 (10.8)	0.1
	<i>papC</i>	3	26	2	1	0.0003*	0	11 (50)	22 (33.8)	1.8
	<i>papG</i>	2	8	2	0	0.1	1	3 (13.6)	9 (13.8)	1.2
	<i>papEF</i>	4	22	1	0	0.001*	0	10 (45.5)	17 (26.2)	0.09
	<i>fimH</i>	20	49	5	4	0.003*	2 (15.4)	22 (100)	54 (83.1)	0.03*
	<i>sfa/foc</i>	0	3	1	0	0.4	0	0	4 (6.2)	0.2
Toxin	<i>bam</i>	1	4	1	0	0.5	0	0	6 (9.2)	0.1
	<i>hlyA</i>	0	0	0	0	N/A	0	0	0	N/A
	<i>vat</i>	1	2	1	0	0.8	0	1 (4.5)	3 (4.6)	1.3
	<i>fyuA</i>	18	37	4	1	0.1	0	21 (95.5)	39 (60)	0.001*
Siderophores	<i>iutA</i>	23	39	4	3	0.6	0	22 (100)	47 (72.3)	0.005*
	<i>ireA</i>	7	20	1	1	0.07*	1 (7.7)	13 (59.1)	15 (23.1)	0.001*
	<i>kpsMII</i>	12	28	3	1	0.3	1 (7.7)	18 (81.8)	25 (38.5)	0.0004*
Protectins	<i>kpsMIII</i>	2	5	0	0	0.3	0	0	9 (13.8)	0.6
	<i>K15</i>	2	6	0	1	0.4	0	0	6 (9.2)	0.1
miscellaneous	<i>rfa</i>	0	0	0	0	N/A	0	0	0	N/A
	<i>ibe10</i>	1	3	0	1	0.8	2	1 (4.5)	2 (3.1)	0.7
	<i>nfa</i>	0	1	0	0	0.3	0	0	1 (1.5)	0.5
pathogenicity islands	<i>PAI</i>	10	18	3	0	0.6	1 (7.7)	11 (50)	19 (29.2)	0.07

*Statistically significant values, N/A; Not applicable, P value was calculated using Chi square test.

ST131 *E. coli* has been prevalent around the world in recent years especially among the resistant strains either fluoroquinolone-resistant *E. coli* (FQ-R) or the extended spectrum beta-lactamase *E. coli* (ESBLs) strains. ST131 clone has emerged as two different subclones; H30 and H30-Rx which were the most common in associating with FQs and ESBLs respectively.

In the current study, 19 of V.Fs have been investigated among 100 *E. coli* isolates that were isolated from different clinical samples including urine (55), exudates (30), sputum (8) and blood (7). The virulence factor scores were significantly higher among ST131 strains for *papC* ($p \leq 0.03$), *fyuA* ($p \leq 0.003$), *iutA* ($p \leq 0.01$), *papEF* ($p \leq 0.01$), and *fimH* ($p \leq 0.05$) than for non ST131 strains. However, all ST131 strains have at least one of virulence factors except one strain which does not have any virulence factors which may harbor other virulence factors that are not included in this study. This result is appropriate with the previous studies that reported that the strains which belonged to ST131 clone had higher virulence scores than non ST131 strains [8, 19-20, 22-23]. Distribution of V.Fs among H30-Rx strains was higher than among non H30-Rx strains. This result approximates with Banerjee et al. [8] for *iutA* (89%) and *kpsMII* (94%). The differences between H30-Rx and non H30-Rx strains were significant for *fimH* ($p \leq 0.03$), *iutA* ($p \leq 0.005$), *fyuA* ($p \leq 0.001$), *ireA* ($p \leq 0.001$) and *kpsMII* ($p \leq 0.0004$). The diversity of the

V.Fs of ST131 strains and its subclones may contribute in its disseminations in different conditions whereas these factors are considered as adhesion factors or as siderophores which are important for bacterial nutrition. This finding can be confirmed by comparing the distribution of the virulence factors among the clinical samples, where we found that the V.Fs were higher among urine samples than other samples, the differences were significantly higher for *papC* ($p \leq 0.0003$), *papEF* ($p \leq 0.001$), *fimH* ($p \leq 0.003$) and *ireA* ($p \leq 0.07$). Most of those factors are important in the adhesion of bacteria on the site of infection which are considered the first step in the bacterial infection. The absence of those factors in other clinical samples may relate to the source of infections which may be caused by commensal strains.

4. Conclusion

High emergence of ST131 and its subclones H30 and H30-Rx among extra-intestinal clinical samples in combination with *CTX-M-15* is considered as a risk indicator that refers to the ability of *E. coli* strains to increase the morbidity and the mortality through its dissemination among commensal strains and into the community. Further studies in different regions are needed to highlight on factors; *fimH*, *iutA*, and *fyuA* which were the highest prevalent among H30-Rx strains.

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