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Original Article

Microbiology Section

Phylogenetic Distribution of Virulence Genes Among ESBL-producing Uropathogenic *Escherichia coli* Isolated from Long-term Hospitalized Patients

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ABSTRACT

Objectives: The present study was aimed to investigate the antibiotic resistance, virulence potential and phylogenetic grouping of ESBL-producing uropathogenic *Escherichia coli* (EP-UPEC) isolated from long-term hospitalized patients.

Materials and Methods: EP-UPEC isolates from September 2013 to June 2014 at a tertiary care hospital of China were screened for ESBL-production by the double disk diffusion test. Isolates with ESBL-phenotype were further characterized by antibiotic resistance testing, PCR of different ESBL and virulence genes, and phylogenetic grouping.

Results: One hundred and twenty EP-UPEC were isolated from long-term hospitalized patients. All EP-UPEC isolates were resistant to Ampicillin, Cefazolin, Cefuroxime, Cefotaxime, Cefoperazone and Ceftriaxone, and the majority of EP-UPEC isolates were resistant to Piperacillin (82.5%), Ciprofloxacin (81.2%), Trimethoprim-Sulfamethoxazole (72.5%). The isolates showed the highest sensitivity against Imipenem (98.4%), Piperacillin/tazobactam (96.7%), Cefoperazone/sulbactam (91.7%), Amikacin (90.8%) and Cefepime (75.8%). Nine different ESBL genotype patterns were observed and *CTX-M* type was the most prevalent ESBL genotype (42.5%, 51/120). Majority of EP-UPEC isolates possess more than one ESBL genes. EP-UPEC isolates belonged mainly to phylogenetic group B2(36.7%) and D(35.0%). The prevalence of *traT*, *ompT*, *iss*, *PAI*, *afa*, *fimH* and *papC* were 75.8%, 63.3%, 63.3%, 60.8%, 40.8%, 19.2% and 6.7%, respectively. The number of virulence genes (VGs) detected was significantly higher in group B2 than in group A (ANOVA, $p<0.001$), group B1(ANOVA, $p= 0.012$) and D (ANOVA, $p<0.001$).

Conclusions: EP-UPEC strains showed multidrug resistance and co-resistance to other non β-lactam antibiotics. *CTX-M* was the most prevalent ESBL genotype and majority of EP-UPEC strains more than one ESBL genes. EP-UPEC strains belonged mainly to phylogenetic group B2 and D, and most of the virulence genes were more prevalent in group B2.

Keywords: ESBL, Phylogenetic groups, Resistance, UPEC, Virulence

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections, and UPEC is the causative pathogen over 50% nosocomial UTI [1].The production of ESBLs is a common resistant mechanism of UPEC [2]. Cases of UTI caused by ESBL-producing *E.coli* are increasing. Among ESBL genes, *CTX-M*, *TEM*, *SHV* and *OXA* are the major clinical concern, and ESBL-producing *E.coli* is prevalent in several countries in Asia region [3-5]. Antibacterial choice is often complicated by multidrug resistance. There is an increasing association between ESBL production and multidrug resistance.

Acquisition of potential virulence factors by UPEC strains might increase their ability to adapt to new niches, contribute to colonization and invasion into host tissues, avoidance to immune responses and acquiring nutrients from the host [6-8].The virulence genes include adhesion (*afa*, *sfa*, *fimH* and *papC*), protectin (*traT* and *iss*), toxin (*cnf1* and *hlyA*) and siderophore (*iutA*), *iucC* and *ompT* (6-8). *Afa* is associated with pyelonephritis, and recurrent and chronic UTI [9]. *Hly* gene is associated with pyelonephritis and plays a role in lysing nucleated host cells and damaging immune cells [10].

E.coli strains are divided into four main phylogenetic groups: A, B1, B2 and D, and the most *E.coli* strains responsible for UTI belong to group B2 and D [11]. The distribution of phylogenetic groups in different geographic regions may vary.

The purpose of this study was to assess correlating antibiotic resistance, virulence potential and phylogenetic groups of ESBL-producing uropathogenic *E.coli* (EP-UPEC) isolated from long-term hospitalized patients.

MATERIALS AND METHODS

Selection of the Strains

A total of 120 ESBL-producing UPEC strains were isolated from long-term hospitalized patients in the First Affiliated Hospital of Soochow University from September 2013 to June 2014. This hospital has 1800 beds and serves a population of 1,000,000 inhabitants in both urban and rural areas. These strains were obtained from urine samples. The presence of ESBL resistance was evaluated using the Ceftazidime and Ceftazidime-Clavulanic Acid (CAC) and the Cefotaxime and Cefotaxime-Clavulanic Acid (CEC) combination disks.

Susceptibility Testing

Antimicrobial susceptibility test for isolates of *Escherichia coli* was performed against trimethoprim-Sulfamethoxazole (30μg), Ampicillin (10μg), Gentamicin (10μg), Cefazolin (CZO), Cefuroxime (30μg), Cefotaxime (30μg), Ceftriaxone (30μg), Cefoperazone (30μg), Ceftazidime (CAZ), Cefepime (30μg), Cefoperazone/sulbactam (75/30μg), Piperacillin (100μg), Piperacillin/tazobactam (100/10μg), Amikacin (30μg), Ciprofloxacin (5μg), Imipenem (10μg) (Oxoid, UK), by the disc diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI-2011).The resistant genes of *SHV*, *TEM*, *CTM-M* and *OXA* were identified. All the primer sequences used have been used in previous studies [12].

DNA Isolation

All isolates were cultured on blood agar and incubated overnight at 37°C. Genomic DNA was isolated from all strains with Wizard

Genomic DNA purification kit (Promega, China), according to the manufacturer's instructions, and used as template for PCR.

Phylogenetic Grouping Typing of Strains and Detection of Virulence Genes

Phylogenetic grouping typing was performed as described previously [11].The genes encoding *Escherichia coli* virulence genes (*traT*, *papC*, *hlyA*, *iutA (aerJ)*, *sfa*, *afa*, *cnf1*, *fimH*, *PAI*, *iucC*, *iss*, and *ompT*), were performed by single PCR as previously reported [13-18]. The primers used in this study are listed in [Table/Fig-1].

STATISTICAL ANALYSIS

All data were analysed using IBM SPSS 21.0 statistical software. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total number of 120 ESBL-producing UPEC (EP-UPEC) were isolated from long-term hospitalized patients. The distribution of the ESBL groups and the resistant profiles of EP-UPEC isolates are summarized in [Table/Fig-2]. The disk diffusion indicated the resistant rates for the EP-UPEC isolates were 100.0% (120/120) for Ampicillin, Cefazolin, Cefuroxime, Cefotaxime, Cefoperazone and Ceftriaxone, and the majority of EP-UPEC isolates were resistant to Piperacillin (82.5%), Ciprofloxacin (81.2%), Trimethoprim-Sulfamethoxazole (72.5%) Gentamicin (54.2%) and Ceftazidime (44.2%). The isolates showed the highest sensitivity against Imipenem (98.4%), Piperacillin/tazobactam (96.7%), Cefoperazone/sulbactam (91.7%), Amikacin (90.8%) and Cefepime (75.8%). Analysis of antibacterial resistant patterns showed that EP-UPEC isolates were more frequently co-resistant to other non-beta lactam classes of antibiotics.

CTX-M, *TEM*, *SHV* and *OXA* were identified in 90.8% (109/120), 40.0% (48/120), 10.8% (13/120) and10.0% (12/120) of EP-UPEC strains, respectively [Table/Fig-2]. Moreover, nine different ESBL genotype patterns were observed amongst them [Table/Fig-3]. *CTX-M* type was the most prevalent ESBL genotype (42.5%, 51/120), and majority of EP-UPEC isolates possess more than one ESBL genes.

All EP-UPEC isolates for the presence of 12 virulence genes (VGs) was tested. Of 120 EP-UPEC isolates, 110(91.7%) were iucC, and 75.8%, 63.3%, 63.3%, 60.8%, 40.8%, 19.2% and 6.7% of isolates were positive for *traT, ompT, iss, PAI, afa, fimH* and *papC*, respectively [Table/Fig-4]. The *cnf1, sfa, intA and hlyA* products were not detected in any of the isolates.

The distribution of phylogenetic types in isolates is shown in [Table/Fig-5]. Among the 120 EP-UPEC strains, A type, B1 type, B2 type and D type were identified in 14.2% (17/120), 14.2% (17/120), 36.7% (44/120) and 35.0% (42/120) of strains, respectively. The EP-UPEC strains belonged mostly to phylogenetic type B2 (36.7%) and D (35.0%). Comparison of resistance number revealed that there were no significant differences among there four phylogroups [Table/Fig-6].The number of VGs detected varied within the phylogroups and was significantly higher in group B2 than in group A (ANOVA, $p<0.001$), group B1(ANOVA, $p= 0.012$) and D (ANOVA, $p<0.001$). Most of the virulence genes were found to be more prevalent in group B2 [Table/Fig-6]. In group B2, *iucC*, *PAI*, *af aompT* and *fimH* were prevalent as compared to the other three groups.

DISCUSSION

This study was conducted to investigate the antibiotic resistance, virulence potential and phylogenetic grouping of EP-UPEC isolated from long-term hospitalized patients. In our research, EP-UPEC isolates showed multidrug resistance or extreme drug resistance to Ampicillin, first-generation Cephalosporin, second-generation Cephalosporin and third-generation Cephalosporin. Moreover, EP-UPEC isolates showed co-resistance to other non β-lactam antibiotics

Primers	Oligonucleotide sequence (5'–3')	Sizes (bp)	Specificity	Reference
ESBLs				
SHV-F	GGTTATGCGTTATATTCGCC	865	*SHV*	[12]
SHV-R	TTAGCGTTGCCAGTGCTC			
TEM-F	ATGAGTATTCAACATTTCCG	868	*TEM*	[12]
TEM-R	CTGACAGTTACCAATGCTTA			
CTX-M-F	ATGTGCAGYACCAGTAARGT	593	*CTM-M*	[12]
CTM-M-R	TGGGTRAARTARGTSACCAGA			
OXA-F	ACACAATACATATCAACTTCGC	814	*OXA*	[12]
OXA-R	AGTGTGTTTAGAATGGTGATC			
phylogentic group				
chuA-F	GACGAACCAACGGTCAGGAT	279	*chuA*	[11]
chuA-R	TGCCGCCAGTACCAAAGACA			
yjaA-F	TGAAGTGTCAGGAGACGCTG	211	*yjaA*	[11]
yjaA-R	ATGGAGAATGCGTTCCTCAAC			
tspE4C2-F	GAGTAATGTCGGGGCATTCA	152	*spE4C2*	[11]
tspE4C2-R	CGCGCCAACAAAGTATTACG			
Virulent genes				
traT-F	GGTGTGGTGCGATGAGCACAG	290	*traT*	[13]
traT-R	CACGGTTCAGCCATCCCTGAG			
papC-F	GACGGCACTGCTGCAGGGTGTGGCG	328	*papC*	[15]
papC-R	ATATCCTTTCTGCAGGGATGCAATA			
hlyA-F	AACAAGGATAAGCACTGTTCTGGCT	1177	*hlyA*	[14]
hlyA-R	ACCATATAAGCGGTCATTCCCGTCA			
iutA-F	ATGAGCATATCTCCGGACG	587	*iutA (aerJ)*	[16]
iutA-R	CAGGTCGAAGAACATCTGG			
sfa-F	CTCCGGAGAACTGGGTGCATCTTAC	410	*sfa*	[14]
sfa-R	CGGAGGAGTAATTACAAACCTGGCA			
afa-F	GCTGGGCAGCAAACTGATAACTCTC	750	*afa*	[14]
afa-R	CATCAAGCTGTTTGTTCGTCCGCCG			
cnf1-F	AAGATGGAGTTTCCTATGCAGGAG	498	*cnf1*	[14]
cnf1-R	TGGAGTTTCCTATGCAGGAG			
fimH-F	TGTACTGCTGATGGGCTGGTC	564	*fimH*	In the study
fimH-R	GGGTAGTCCGGCAGAGTAACG			
PAI-F	GGACATCCTGTTACAGCGCGCA	930	*PAI*	[17]
PAI-R	TCGCCACCAATCACAGCCGAAC			
iucC-F	AAACCTGGCTTACGCAACTGT	269	*iucC*	[15]
iucC-R	ACCCGTCTGCAAATCATGGAT			
iss-F	GTGGCGAAAACTAGTAAAACAGC	760	*iss*	[18]
iss-R	CGCCTCGGGGTGGATAA			
ompT-F	ATCTAGCCGAAGAAGGAGGC	559	*ompT*	[18]
ompT-R	CCCGGGTCATAGTGTTCATC			

[Table/Fig-1]: Primers used for amplification of ESBL, Phylogenetic grouping and Virulence genes

Antibiotics	Resistant (%)
Ampicillin	100.0
Cefazolin	100.0
Cefuroxime	100.0
Cefotaxime	100.0
Ceftriaxone	100.0
Cefoperazone	100.0
Trimethoprim-Sulfamethoxazole	87 (72.5)
Ciprofloxacin	98 (81.2)
Gentamicin	65 (54.2)
Cefepime	29 (24.2)
Ceftazidime	53 (44.2)
Cefoperazone/sulbactam	10 (8.3)
Piperacillin	99 (82.5)
Piperacillin/tazobactam	4 (3.3)
Amikacin	11 (9.2)

Antibiotics	Resistant (%)
Imipenem	2(1.6)
ESBL genes	
CTX-M	109(90.8)
TEM	48(40.0)
SHV	13(10.8)
OXA	12(10.0)

[Table/Fig-2]: Drug resistance and ESBLs of EP-ESBLs

ESBL genotypes	No. of isolates
CTX-M,TEM,SHV	6 (5.0)
CTX-M,TEM	36 (30.0)
CTX-M,SHV	6 (5.0)
CTX-M,OXA	10 (8.3)
TEM,OXA	1 (0.8)
CTX-M	51 (42.5)
TEM	5 (4.2)
SHV	1 (0.8)
OXA	1 (0.8)

[Table/Fig-3]: Distribution of ESBL genotypes in EP-UPEC isolates

virulence genes	No. of isolates
iucC	110(91.7)
PAI	73(60.8)
fimH	23(19.2)
afa	49(40.8)
traT	91(75.8)
ompT	76(63.3)
iss	76(63.3)
papC	8(6.7)

[Table/Fig-4]: Distribution ofVirulence genes in EP-UPEC isolates

Phylogroups	No. of isolates
A	17(14.2)
B1	17(14.2)
B2	44(36.7)
D	42(35.0)

[Table/Fig-5]: Distribution of Phylogroups in EP-UPEC isolates

Resistance pattern	A(n=17)	B1(n=17)	B2(n=44)	D(n=42)	Total(n=120)
Trimethoprim-Sulfamethoxazole	14(82.4)	16(94.1)	32(72.7)	25(59.5)	87(72.5)
Ciprofloxacin	13(76.5)	14(82.4)	34(77.3)	37(88.1)	98(81.2)
Gentamicin	11(64.7)	7(41.2)	23(52.3)	24(57.1)	65(54.2)
Cefepime	8(47.1)	2(11.8)	7(15.9)	12(28.6)	29(24.2)
Ceftazidime	12(70.6)	9(52.7)	14(31.8)	18(42.9)	53(44.2)
Cefoperazone/ sulbactam	1(5.9)	1(5.9)	4(9.1)	4(9.5)	10(8.3)
Piperacillin	14(82.4)	15(88.2)	33(75.0)	37(88.1)	99(82.5)
Piperacillin/ tazobactam	2(11.8)	1(5.9)	0	1(2.4)	4(3.3)
Amikacin	2(11.8)	3(17.6)	2(4.5)	4(9.5)	11(9.2)
Imipenem	0	1(5.9)	1(2.3)	0	2(1.6)
virulence genes					
iucC	16(94.1)	14(82.4)	44(100.0)	36(85.7)	110(91.7)
PAI	3(17.6)	6(35.3)	42(95.5)	22(52.4)	73(60.8)
fimH	2(11.8)	2(11.8)	13(29.5)	6(14.3)	23(19.2)
afa	8(47.1)	6(35.3)	24(54.5)	11(26.2)	49(40.8)
traT	9(52.9)	15(88.2)	31(70.5)	36(85.7)	91(75.8)
ompT	7(41.2)	10(58.8)	41(93.2)	18(42.9)	76(63.3)
iss	9(52.9)	14(82.4)	21(47.7)	32(76.2)	76(63.3)
papC	0	0	3(6.8)	5(11.9)	8(6.7)

[Table/Fig-6]: Distribution of resistance pattern and virulence genes in groups A, B1, B2 and D

like Ciprofloxacin, Piperacillin, Trimethoprim-Sulfamethoxazole and Gentamicin. Conversely, highest susceptibility was found to Imipenem (98.4%), Piperacillin/tazobactam (96.7%), Cefoperazone/ sulbactam (91.7%), Amikacin (90.8%) and Cefepime (75.8%). Other studies also demonstrated that ESBL-producing *E.coli* strains were high resistant Ciprofloxacin, Piperacillin, Trimethoprim-Sulfamethoxazole and Gentamicin, and higher susceptive to carbapenems and Amikacin [19-21].

ESBL genotyping results showed that UPEC isolates carried different type ESBL genes, and 90.8% were *CTX-M*-positive. Moreover, nine different ESBL genotype patterns were observed amongst them. Similar to other studies [22-24], we found that *CTX-M* type was the most prevalent ESBL genotype (42.5%, 51/120), and majority of EP-UPEC isolates possess more than one ESBL genes. Therefore, the possible role of these genes either alone or in combination for ESBL cannot be ruled out. *E.coli* strains are divided into four main phylogenetic groups designed A, B1, B2 and D, and the most *E.coli* strains responsible for UTI belong to group B2 and D [11]. In the study, phylogenetic grouping revealed that EP-UPEC isolates belonged mainly to phylogenetic group B2 (36.7%) and D (35.0%). As reported previously, most of the uropathogenic *E.coli* isolates belonged to the phylogenetic group B2, D [25] and most of VGs were more prevalent in phylogenetic group B2 and/or D [26-29]. In the study, the number of VGs detected varied within the phylogroups and was significantly higher in group B2 than in other three groups. Most of the virulence genes were found to be more prevalent in group B2, which is concordant with previous studies [26,27,29]. In group B2, *iucC*, *PAI*, *afa, ompT* and *fimH* were prevalent than in the other three groups, concordant with previous studies [30]. These results indicate that virulent and pathogenic *E.coli* isolates are usually associated with phylogenetic group B2.

CONCLUSION

This study indicates that EP-UPEC strains show multidrug resistance, and co-resistance to other non β-lactam antibiotics. *CTX-M* is the most prevalent ESBL genotype and majority of EP-UPEC strains more than one ESBL genes. EP-UPEC strains belong mainly to phylogenetic group B2 and D, and most of the virulence genes are more prevalent in group B2.These results suggest that resistance, virulence and phylogenetic groups are three different mechanisms for the outcome of EP-UPEC infection. Phylogenetic distribution of virulence genes among ESBL-producing uropathogenic *E.coli* isolated from long-term hospitalized patients in the study enhanced our current knowledge of the resistance, the pathogenicity and genetic characteristics of EP-UPEC. Moreover, determining the correlation of resistance, virulence and phylogenetic groups is crucial for the prevention and control of nosocomial UTI caused by ESBL-producing *E.coli*.

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