

# 20-5960 Whole genome analysis and phylogenetic characteristics of Escherichia coli isolated from urine and stones in calcium oxalate stone patients

Fangling Zhong, Dong Chen, Weizhou Wu, Yongchang Lai, Hans-Göran Tiselius, Chonghe Jiang, Jinkun Huang, Xiaolu Duan, Simon Choong, Yeping Liang, Guohua Zeng, Ming Lei, Wenqi Wu

## Abstract

Previous clinical studies have shown that Escherichia coli (E. coli) is the most common strain of bacteria isolated from urine and stones in patients with calcium oxalate (CaOx) stone disease. The lesson learnt so far is that E coli might serve as a bridge between urine and stones. But whether bacteria in stones are derived from bacteria in urine and whether there are differences between bacteria in stones and urine in terms of virulence genes or genetic background remain unclear.

## Objective

To get some further insights of the relationships and possible differences in characteristics between ESC and EUC in terms of antimicrobial susceptibility, phylogenetic grouping and genotype. Whole genome sequencing and comparative genome analysis were carried out in order to further identify genome features and genetic background of isolated E. coli strains. All these analyses were carried out as an attempt to increase our knowledge of the variability in properties of E. coli important for urinary tract pathology.

## Methods

Totally 83 bacterial isolates (33 EUC and 50 ESC) from 66 patients with CaOx stone disease were included in the study. Comparison of characteristics and relationships between E.coli in urine and stones, were assessed by antimicrobial susceptibility test, phylogenetic grouping and genotyping. In addition, whole genome sequencing and comparative genomic analysis were carried out in 16 paired ESC and EUC isolated strains from 8 patients.

## Results

Antimicrobial susceptibility tests revealed that the E. coli strains were multidrug resistant (MDR), and that ESC and EUC had the same or similar pattern of resistant genes. The most common phylogenetic group was B2, recorded in 54.0% of the ESC samples and 69.7% of the EUC samples. E.coli isolated from stones and urine from the same patients shared virulence genes that were highly homologous and largely consistent. Moreover, these E.coli strains were located in the same clade and may thus originate from a common ancestor.

Table 1. Distribution of antimicrobial resistance in E.coli strains isolated from urine (EUC) and stones (ESC).

Antimicrobial	ESC(n=50)		EUC(n=33)		p
	R/No	%	R/No	%	
Amikacin	2/50	4.0	2/33	6.1	1.0
Amoxicillin/Clavulanate	5/50	10.0	4/33	12.1	1.0
Ampicillin	29/37	78.4	10/12	83.3	1.0
Aztreonam	13/36	36.1	5/12	41.7	0.743
Cefazolin	21/49	42.9	20/31	64.5	0.059
Cefepime	14/49	28.6	9/32	28.1	0.965
Cefoperazone/Sulbactam	4/47	8.5	1/32	3.1	0.621
Cefotaxime	24/50	48.0	18/33	54.6	0.559
Cefoxitin	6/48	12.5	6/33	18.2	0.479
Ceftazidime	13/47	27.7	11/33	33.3	0.586
Ceftriaxone	24/49	49.0	18/33	54.6	0.621
Cefuroxime	23/46	50.0	14/33	42.4	0.506
Ciprofloxacin	15/37	40.5	5/12	41.7	0.945
Ertapenem	2/50	4.0	0/33	0	0.515
Gentamicin	15/37	40.5	9/12	75.0	0.081
Imipenem	2/50	4.0	0/33	0	0.515
Levofloxacin	20/48	41.7	13/33	39.4	0.838
Meropenem	2/47	4.3	0/33	0	0.509
Nitrofurantoin	4/45	8.9	0/24	0	0.289
Piperacillin/Tazobactam	3/49	6.1	2/32	6.3	0.981
Sulfamethoxazole	28/50	56.0	17/33	51.5	0.688
Tobramycin	4/35	11.4	5/12	41.7	0.061

R: resistance; No: number.

Table 2. Distribution on phylogenetic groups and sequence types (ST) in 83 E.coli isolates. Number (%).

	Total (n=83)	ESC (n=50)	EUC (n=33)	p
<b>Phylogenetic group</b>				
B2	50(60.2)	27(54.0)	23(69.7)	0.153
D	25(30.1)	16(32.0)	9(27.3)	0.646
A	6(7.2)	5(10.0)	1(3.0)	0.443
B1	2(2.4)	2(4.0)	0	0.666
<b>Sequence type (ST)</b>				
ST1193	15(18.1)	6(12.0)	9(27.3)	0.077
ST131	11(13.3)	6(12.0)	5(15.2)	0.933
ST73	6(7.2)	3(6.0)	3(9.1)	0.921
ST69	5(6.0)	4(8.0)	1(3.0)	0.646
ST12	4(4.8)	3(6.0)	1(3.0)	0.925
ST224	4(4.8)	4(8.0)	0	0.254
ST648	4(4.8)	3(6.0)	1(3.0)	0.925
ST62	3(3.6)	1(2.0)	2(6.1)	0.712
ST156	3(3.6)	2(4.0)	1(3.0)	1.0
ST162	3(3.6)	2(4.0)	1(3.0)	1.0
ST6756	2(2.4)	2(4.0)	0	0.151
ST405	2(2.4)	1(2.0)	1(3.0)	1.0
ST550	2(2.4)	1(2.0)	1(3.0)	1.0
ST3177	2(2.4)	1(2.0)	1(3.0)	1.0
ST4381	2(2.4)	1(2.0)	1(3.0)	1.0
ST6807	2(2.4)	1(2.0)	1(3.0)	1.0
other	13(15.7)	9(18.0)	4(12.1)	0.68

EUC: E.coli isolated from urine.

ESC: E.coli isolated from stones

Table 3. General characteristics of 16 whole genome sequenced isolates of E. coli from urine (EUC) and stones (ESC) in 8 patients.

Patient No.	Isolate	Source	ST	Raw Data (Mb)	Genome Size (Mb)	%GC content	No. of contigs (>500 bp)	N50 Length(bp)	Total Length(bp)	GenBank accession no
1	ESC1	stone	1193	848	5.68	50.60	41	237,323	4,934,974	SAMN09829079
	EUC1	urine	1193	861	5.62	50.45	59	233,866	5,032,283	SAMN09829087
2	ESC2	stone	1193	921	5.87	50.60	64	222,076	5,090,175	SAMN09829080
	EUC2	urine	1193	861	5.55	50.59	69	222,466	5,105,378	SAMN09829088
3	ESC3	stone	1193	859	5.61	50.59	66	207,532	5,106,238	SAMN09829081
	EUC3	urine	1193	841	5.51	50.54	70	210,541	5,189,592	SAMN09829089
4	ESC4	stone	1193	862	5.35	50.63	52	222,472	4,988,410	SAMN09829082
	EUC4	urine	1193	862	5.39	50.61	67	222,076	5,060,440	SAMN09829090
5	ESC5	stone	1193	855	5.84	50.43	62	201,068	5,054,441	SAMN09829083
	EUC5	urine	1193	859	6.13	50.58	70	216,510	5,095,647	SAMN09829091
6	ESC6	stone	131	845	5.3	50.77	64	353,825	5,120,644	SAMN09829084
	EUC6	urine	131	860	5.35	50.76	59	353,825	5,110,513	SAMN09829092
7	ESC7	stone	131	859	5.0	50.73	66	238,968	4,945,649	SAMN09829085
	EUC7	urine	131	847	5.04	50.74	69	238,458	4,951,026	SAMN09829093
8	ESC8	stone	131	865	5.89	50.68	82	248,754	5,305,291	SAMN09829086
	EUC8	urine	131	844	5.8	50.69	87	239,941	5,323,603	SAMN09829094

## Conclusions

ESC and EUC isolated from patients with CaOx stones had a high prevalence of phylogenetic groups. Bacterial strains isolated from urine and stones in the same patient had consistent antimicrobial susceptibility profiles, phylogenetic groups, genotypes, virulence genes, resistance genes, high sequence co-linearity and close relationships.