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Antibiotic-resistant ST38, ST131 and ST405 strains are the leading uropathogenic *Escherichia coli* clones in Riyadh, Saudi Arabia

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Objectives: We investigated the molecular epidemiology of uropathogenic *Escherichia coli* (UPEC) from a tertiary care hospital in Riyadh, Saudi Arabia, revealing, for the first time, the population structure of UPEC in the region.

Methods: A total of 202 UPEC isolates were recovered from hospital and community patients with urinary tract infection in December 2012 and January 2013. Strains were characterized by MLST, antibiotic susceptibility determination and virulence gene detection.

Results: The most common lineages were ST131 (17.3%), ST73 (11.4%), ST38 (7.4%), ST69 (7.4%), ST10 (6.4%), ST127 (5.9%), ST95 (5.4%), ST12 (3.5%), ST998 (3.5%) and ST405 (3%). ST131 and ST405 isolates were significantly associated with high levels of antibiotic resistance (60% of ST131 carried CTX-M-14 or CTX-M-15 and 66.7% of ST405 isolates carried CTX-M-15). ST131, CTX-M-15-positive isolates were predominantly of the *fimH*30/clade C group, resistant to fluoroquinolones; members of this sub-group were more likely to carry a high number of genes encoding selected virulence determinants. The relatively high proportion of ST38 was notable and four of these isolates harboured *aggR*.

Conclusions: Our findings highlight the presence of MDR, CTX-M-positive ST38, ST131 and ST405 UPEC in Saudi Arabia. The high proportion of isolates with CTX-M is a particular concern. We suggest that ST38 UPEC warrant further study.

Introduction

Urinary tract infection (UTI), one of the most common infections in humans, is predominantly caused by uropathogenic *Escherichia coli* (UPEC). The population structure and antibiotic susceptibility of leading UPEC clones and the importance of key virulence-associated traits and distinctive O antigens in pathogenesis have been widely studied.[1,2]

Globally disseminated MDR strains of O25b:H4-ST131 *E. coli* (ST131) are responsible for a high proportion of UTI and bloodstream infections.[3] The most prevalent ST131 sub-clone is associated with widespread dissemination of the CTX-M-15 ESBL enzyme with co-resistance to fluoroquinolones and carriage of the H30 variant of the type 1 fimbrial adhesin gene, *fimH*.[4] This sub-clone is known as *H30*-Rx, or clade C.[3,5] Although UPEC are extraintestinal *E. coli* (ExPEC) pathogens, some strains carry virulence properties more characteristic of enteroaggregative *E. coli* (EAEC).[6,7]

To our knowledge, there have been no robust epidemiological studies of UPEC in Saudi Arabia, a key member state of the Middle East and the Gulf Cooperation Council. A recent systematic review shows a growing problem of antibiotic-resistant Gram-negative bacteria in this region, but the roots of the problem have never been fully investigated.[8] Accordingly, we have examined the population structure of UPEC at a tertiary care hospital in Saudi Arabia and our findings may help inform infection control to prevent the spread of MDR strains from the region.

Materials and methods

Bacterial isolates

A total of 202 non-duplicate, consecutive isolates of *E. coli* from patients with clinically confirmed UTI were obtained from King Abdulaziz Medical City (KAMC) in Riyadh, Saudi Arabia. This 1000 bed tertiary healthcare centre serves a population of 500 000 of the Saudi National Guard soldiers and

their dependants. Prospectively collected isolates from inpatients and outpatients (December 2012 to January 2013) were not selected on the basis of any resistance profile. Identification was performed using the Vitek II XL (bioMérieux, France) with the Gram Negative Susceptibility Card, AST-GN26. Isolates were maintained on Sheep Blood Agar (Saudi Prepared Media Laboratory) and Transwabs® in Amies transport medium (Medical Wire and Equipment, England) were used to transport the isolates to the UK, following international guidelines (UN 2814). Clinical and demographic parameters (patient age, gender, specimen type and geographical origin) were examined anonymously.

Molecular typing

Pure cultures on Columbia Agar (Oxoid), incubated at 37°C for 18 h, were used for DNA extraction with the PrepMan Ultra kit (Applied Biosystems, USA). MLST was carried out using the Achtman scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).[9] *fimH* typing was performed to investigate possible sub-typing within STs.[4] Molecular serotyping was performed to detect 14 serogroups associated with UTI.[10] Additional O typing was performed on all ST131 strains to detect the O16-ST131 and O25b-ST131 clades.[5]

Virulence genotyping

Virulence factor (VF) carriage was assessed by PCR.[1] The virulence score equalled the sum of positive VFs for each isolate. All ST38 isolates were screened for the presence of the EAEC transport regulator gene (*aggR*)[6] in 25 μL reactions containing 12.5 μL of 2× BioMix Red (Bioline USA Inc.), 1 μL of each primer (10 pmol/μL) and 1 μL of chromosomal DNA. Amplification proceeded at 94°C for 4 min, 30 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were visualized with UV transillumination in agarose gels.

Antimicrobial susceptibility profiling

Susceptibility testing was performed using the Vitek II XL with AST-GN26 cards. Resistance scores were calculated by dividing the number of antibiotics to which an isolate was resistant by the total number of antibiotics being studied. Selected isolates were screened for the presence of β-lactamase genes bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM-1} and $bla_{OXA-1/48}$.[11–14] Strains from STs associated with quinolone resistance were examined for mutations in quinolone resistance-determining regions of *gyrA* and *parC*.[15]

Statistical analysis

Fisher's exact test was conducted with a threshold for statistical significance of $P<0.05$.

Results and discussion

Molecular typing reveals the population structure of UPEC in Riyadh

The majority of examined isolates (87.1%) were from females and patient age ranged from <2 months to 87 years (mean age 40 years); 141 isolates (69.2%) were considered to be community acquired, being recovered from outpatients with a clinical diagnosis of uncomplicated UTI.

MLST resolved 51 unique STs. The most common were ST131 ($n=35$; 17.3% of isolates), ST73 (23; 11.4%), ST38 (15; 7.4%), ST69 (15; 7.4%), ST10 (13; 6.4%), ST127 (12; 5.9%) and ST95 (11; 5.4%), which accounted for 61% of the isolates (Table S1, available as Supplementary data at *JAC* Online). On the whole, this reflects the global dominance of a small number of lineages, usually including ST131.[3,15,16] Members of ST131 were predominantly community isolates (69%; 24/35 isolates) and the majority (77%; 27/35) belonged to O25b-ST131 *fimH30*/clade C[3]; 20% (7/35) were from O16-ST131 *fimH41* and one was O25b-ST131 *fimH22*.[5,17]

ST38 isolates have not been widely reported as predominant members in collections of UPEC, but it has been suggested that this group is evolving and is becoming more commonly seen in UTI.[6,18] The diversity in VF carriage and susceptibility profiles observed here (see below) suggests that several ST38 strains are circulating in the population in Riyadh.

High levels of antibiotic resistance are present in the KAMC isolates

STs varied considerably in antibiotic susceptibility from the most susceptible, ST73 (resistance score median 0.1 and range 0.0–0.3), to the most resistant, ST131 (median 0.5 and range 0.2–0.7). Overall, 35% of the isolates exhibited an ESBL phenotype (Table 1). Similar levels of ESBL-producing UPEC have been reported in some countries in the Asia-Pacific region and the Middle East.[8,19] However, in the EU and North America, reported rates are often close to, or below, 5%.[15,16] Many ESBL producers were co-resistant to non-β-lactam antibiotics, including ciprofloxacin and trimethoprim/sulfamethoxazole, which is a cause for concern, as this will lead to limited treatment options for UPEC infection. The high prevalence of ESBLs in Riyadh is already driving high levels of carbapenem prescriptions in the community (S. M. Al Johani, KAMC), which is a genuine threat to the future clinical utility of these antibiotics.

ST131 ($P\leq0.002$) and ST405 ($P\leq0.02$) were significantly associated with ESBL production (Table 1). Although there was not a significant association, 5/7 ST998 isolates were also ESBL producers. This ST has not been widely reported in UTI, but may be an important resistant clone in Riyadh. Detection of CTX-M-like genes was conducted to investigate the mechanism of ESBL production in ST38, ST131 and ST405, as these key clones demonstrated elevated levels of ESBL production. In line with previous reports, CTX-M carriage was most common in ST131 isolates ($n=21$ 60% of ST131) that were positive for CTX-M-14 (eight isolates belonging to O25b-ST131 *fimH30*) and CTX-M-15 (nine isolates belonging to O25b-ST131 *fimH30* and two isolates from O16-ST131 *fimH41*).[2,5] One O25b-ST131 *fimH30* isolate carried both CTX-M-14 and CTX-M-15. The ESBL phenotype of ST38 and ST405 isolates was predominantly mediated by CTX-M-15 (5/6 and 4/5 isolates, respectively). Little is known about the spread of CTX-M-type ESBLs among EAEC strains,[6] but the surprisingly high detection rate for ST38 UPEC in our study and the suggested emergence of ExPEC with UPEC and EAEC characteristics indicates that increased surveillance for this clone is warranted, so that we can fully understand the significance of these pathotypes in UTI. A total of 10 isolates from O25b-ST131 *fimH30* and 5 isolates of ST38 were positive for OXA-1-like determinants.

Resistance to fluoroquinolones was significantly associated with ST131, ST405 and ST410 (Table 1). The O25b-ST131 *fimH30*/clade C isolates were significantly more likely to be ciprofloxacin-resistant ($P\leq0.0008$) than members of O16-ST131 *fimH41*. Most of the ciprofloxacin-resistant isolates had multiple mutations in both *gyrA* and *parC* (Table S2), which has been

Table 1. Association between MLST lineage and resistance to different antibiotic agents (numbers of resistant isolates are given in parentheses)

		β-Lactam										Aminoglycoside		Quinolone		Furane		
ST	Isolates (*n*)	AMP (153)	AMC (18)	PIP (153)	CEF (90)	CXM (68)	FOX (19)	CPD (68)	CTX (62)	CAZ (37)	ESBL (71)	AAC(3), ANT(2) (26)	AAC(6) (15)	CIP (62)	NOR (88)	NIT (9)	Trimethoprim/ sulfamethoxazole (105)	Resistance score [mean, median (range)]
ST131	35	33	2	33	27	20	0	20	20	14	21	9	4	25	32	4	23	0.43, 0.5 (0.2–0.7)
P value		0.007	—	0.007	0.01	0.006	—	0.003	0.001	0.0008	0.002	0.014	—	0.0001	0.0001	—	—	
ST73	23	15	1	15	7	0	0	0	0	0	0	0	0	0	0	0	9	0.11, 0.1 (0.0–0.3)
P value		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
ST38	15	15	4	15	8	6	2	6	6	3	6	1	2	3	8	3	8	0.33, 0.3 (0.1–0.7)
P value		0.02	—	0.02	—	—	—	—	—	—	—	—	—	—	—	0.02	—	
ST69	15	14	2	14	4	4	2	4	3	1	4	1	0	1	2	0	12	0.23, 0.2 (0.1–0.7)
P value		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	
ST10	13	8	1	8	3	3	1	3	2	0	3	0	0	2	2	0	7	0.17, 0.2 (0.0–0.5)
P value		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
ST127	12	7	0	7	3	1	0	1	1	0	1	0	0	0	0	0	2	0.1, 0.1 (0.0–0.4)
P value		—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	
ST95	11	5	1	5	2	2	1	2	1	2	2	0	0	1	3	0	3	0.14, 0.1 (0.0–0.5)
P value		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
ST12	7	3	0	3	1	1	0	1	1	0	1	1	0	1	4	0	4	0.16, 0.2 (0.0–0.5)
P value		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
ST998	7	6	2	6	5	5	2	5	5	2	5	3	0	0	0	0	3	0.38, 0.4 (0.0–0.7)
P value		—	—	—	—	0.02	—	0.05	0.04	—	—	0.017	—	—	—	—	—	
ST405	6	6	0	6	6	5	4	5	5	4	5	0	0	6	6	0	6	0.53, 0.6 (0.4–0.6)
P value		—	—	—	0.04	0.005	0.001	0.02	0.01	0.009	0.02	—	—	0.0008	0.007	—	0.03	
ST410	6	6	2	6	2	2	1	2	2	2	2	0	1	5	6	0	4	0.39, 0.2 (0.2–0.8)
P value		—	—	—	—	—	—	—	—	—	—	—	—	0.01	0.007	—	—	

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; PIP, piperacillin; CEF, cefalotin; CXM, cefuroxime; FOX, cefoxitin; CPD, cefpodoxime; CTX, cefotaxime; CAZ, ceftazidime; AAC, *N*-acetyltransferase; ANT, *O*-adenyltransferase; CIP, ciprofloxacin; NOR, norfloxacin; NIT, nitrofurantoin.

P values (by Fisher's exact test) are shown when $P \leq 0.05$ and these relate to differences found when susceptibility profiles for isolates of each ST were compared with those of all other STs combined.

Table 2. Prevalence of various UPEC-associated VFs within the most commonly detected clonal groups observed in the isolate collection

		Prevalence (%) of VF by ST (no. of isolates is given in parentheses)								
Category	Specific trait	total ($n=202$)	ST10 ($n=13$)	ST38 ($n=15$)	ST69 ($n=15$)	ST73 ($n=23$)	ST95 ($n=11$)	ST127 ($n=12$)	ST131 ($n=35$)	ST405 ($n=6$)
Adhesion	*papA*	59 (29)	5 (38)	3 (20)	**8 (53)**	8 (35)	6 (55)	**7 (58)**	8 (23)	2 (33)
	papC	72 (36)	6 (46)	4 (27)	**10 (67)**	8 (35)	5 (45)	7 (58)	11 (31)	0 (0)
	papEF	56 (28)	2 (15)	2 (13)	**9 (60)**	**12 (52)**	5 (45)	**8 (67)**	5 (14)	1 (17)
	papGII,III	15 (7)	1 (8)	0 (0)	0 (0)	1 (4)	**6 (55)**	**3 (25)**	3 (9)	0 (0)
	allele-II	39 (19)	2 (15)	4 (27)	**7 (47)**	6 (26)	3 (27)	3 (25)	**2 (6)**	1 (17)
	allele-III	42 (21)	5 (38)	3 (20)	2 (13)	3 (13)	3 (27)	4 (33)	10 (29)	1 (17)
	sfa/foc DE	34 (17)	1 (8)	2 (13)	2 (13)	3 (13)	**5 (45)**	2 (17)	8 (23)	1 (17)
	afa/draBC	25 (12)	0 (0)	2 (13)	0 (0)	1 (4)	1 (9)	**4 (44)**	4 (11)	1 (17)
	sfaS	22 (11)	0 (0)	0 (0)	1 (7)	2 (9)	**4 (36)**	3 (25)	5 (14)	1 (17)
Toxins	*hlyA*	14 (7)	0 (0)	1 (7)	0 (0)	3 (13)	**4 (36)**	2 (17)	0 (0)	0 (0)
	cnf1	42 (21)	2 (15)	4 (27)	1 (7)	**10 (43)**	4 (36)	4 (33)	9 (26)	0 (0)
Siderophore	*fyuA*	154 (76)	8 (62)	13 (87)	11 (73)	19 (83)	**4 (36)**	9 (75)	30 (86)	6 (100)
	iutA	118 (58)	5 (38)	10 (67)	11 (73)	15 (65)	8 (73)	7 (58)	25 (71)	1 (17)
Capsule	*kpsM II*	76 (38)	4 (31)	4 (27)	8 (53)	9 (39)	6 (55)	6 (50)	**20 (57)**	1 (17)
	K1	30 (15)	3 (23)	1 (7)	2 (13)	2 (9)	1 (9)	3 (25)	8 (23)	1 (17)
	K5	75 (37)	2 (15)	4 (27)	6 (40)	10 (43)	4 (36)	**9 (75)**	14 (40)	1 (17)
Miscellaneous	*cvaC*	6 (3)	0 (0)	0 (0)	2 (13)	0 (0)	**2 (18)**	1 (8)	0 (0)	0 (0)
	traT	128 (63)	4 (31)	10 (67)	**15 (100)**	17 (74)	7 (64)	**12 (100)**	21 (60)	2 (33)
	PAI (*malX*)	65 (32)	2 (15)	7 (47)	**9 (60)**	10 (43)	6 (55)	4 (33)	13 (37)	2 (33)
VF score [mean, median (range)]			5, 5 (1–11)	6.2, 5 (2–13)	8.1, 7 (2–14)	7.9, 7 (2–15)	9.2, 10 (2–14)	9.4, 9.5 (5–14)	7.1, 6 (2–13)	4.8, 4 (2–10)

Bold values indicate significant associations ($P \leq 0.05$); underlining indicates a negative association.

reported previously.[15,17] However, two fluoroquinolone-resistant isolates from O25b-ST131 *fimH30*/clade C and ST410 showed E(153)K mutations in *gyrA*, which has only been previously described on a single occasion in *Pseudomonas aeruginosa*.[20]

Prevalence of VFs varies widely across ST

STs varied considerably in VF content, from ST405, with the lowest score (mean 4.8), to ST127, with the highest score (mean 9.4) (Table 2). Members of ST131 showed a moderate VF score (mean 7.1), which was equivalent to VF carriage across other phylogenetic group B2 clones (e.g. ST73, ST95 and ST127 in Table S1).

In isolates from ST73, ST95 and ST127, carriage of several VFs was lower than expected.[1,15] These included kpsM II, K1 and PAI (*malX*) and the variation may indicate geographical differences in VF carriage, an interesting observation that warrants further study (Table 2).

Plasmid-mediated carriage of the EAEC transport regulator gene, *aggR*, has been reported as a key feature of emerging ST38 UPEC from Germany, the Netherlands and the UK.[6] Accordingly, ST38 isolates were screened for *aggR* and 4/15 were positive (Table S3), though we did not examine plasmid carriage in these isolates. Overall, ST38 isolates varied in VF carriage from 2 to 13, with no clear differentiation between VF carriage in *aggR*-positive or *aggR*-negative strains (Table S3). The presence of *aggR* may indicate the evolution of a strain possessing both UPEC and EAEC characteristics and highlights the diverse mechanisms employed by *E. coli* to ensure fitness and mediate pathogenicity in different ecological niches. On-going genome sequence analysis of some ST38 strains from Riyadh will significantly enhance our understanding of these organisms.

Conclusions

In conclusion, following the recent report of carbapenem-resistant NDM-1 and OXA-48 *E. coli* strains in the Arabian region,[8] we now describe the population structure of UPEC from a major medical centre in Saudi Arabia, revealing the presence of MDR strains with high virulence potential, including those of ST131. ST38 strains from the region carry both UPEC and EAEC virulence determinants and warrant close monitoring.

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Transparency declarations

None to declare.

Supplementary data

Tables S1–S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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