Persisting clones of *Escherichia coli* isolates from recurrent urinary tract infection in men and women

Urinary tract infection (UTI) is one of the most frequent bacterial infections worldwide (Weichhart *et al.*, 2008) and a common cause of morbidity, especially in women (Foxman & Brown, 2003).

Escherichia coli is the main aetiological agent of UTI (Foxman & Brown, 2003). Despite the frequent recurrence of UTIs caused by *E. coli*, little is known about the bacterial characteristics associated with recurrent urinary tract infection (RUTI); the pathogenesis is incompletely described and not definitively explained. In the past it was believed that RUTI was primarily due to reinfection with a new strain (Brauner *et al.*, 1992; Foxman *et al.*, 2000). However, since the more widespread application of PFGE typing, the hypothesis of RUTIs being caused by persistence or relapse is now more widely accepted (Ejrnæs *et al.*, 2006; Køljalg *et al.*, 2009). The terms used in this study for UTI occurring repeatedly, modified from those of Ejrnæs *et al.* (2006), are as follows: recurrence is used for repeatedly occurring UTI without knowledge of strain similarity; reinfection is used for UTI with a new strain, i.e. a strain different from the primary strain; persistence is used for UTI with the same strain as the primary infecting strain with no known negative culture separating the urine cultures; and relapse is used for UTI with the same strain as the primary infecting strain but with a negative urine culture or a culture different from the primary infecting strain separating the two urine cultures. The aim of the study was to identify and characterize consecutive isolates from cases of RUTI to investigate whether RUTI caused by *E. coli* is primarily due to persistence or reinfection. We also aimed to generate further knowledge about the characteristics of *E. coli* associated with RUTI.

Between December 2005 and April 2006, consecutive urinary culturing on patients with symptoms of UTI was performed at a general practitioner clinic (not associated

to any hospital) based south of Copenhagen. The clinic consisted of nine general practitioners serving approximately 10 800 inhabitants. During the study period, a total of 131 UTI *E. coli* isolates were collected from 102 patients with community-acquired UTI (Jakobsen *et al.*, 2010a; Trobos *et al.*, 2008). From 13 of the patients (10 women and 3 men), more than one *E. coli* isolate ($n=41$) was obtained with 2–7 isolates per patient (Table 1); these patients were categorized as having RUTI. The interval between two consecutive urine cultures was 7–60 days. The patients were aged 17–81 years (median age 60 years).

The MIC of ampicillin, ciprofloxacin, gentamicin, sulfamethoxazole, tetracycline, trimethoprim and nitrofurantoin was determined for the isolates. Results were interpreted according to European Committee on Antimicrobial Susceptibility Testing guidelines (www.eucast.org), except for sulfamethoxazole and tetracycline, which were interpreted according to CLSI (2008). For ciprofloxacin a break-point of ≥ 0.125 mg l⁻¹ was used (Aarestrup *et al.*, 2003). The 41 isolates were PFGE typed using the Pulse-Net database (Hunter *et al.*, 2005; Ribot *et al.*, 2001). The phylogenetic background (A, B1, B2, D and non-typable) of the *E. coli* isolates was determined by PCR (Jakobsen *et al.*, 2010a; Gordon *et al.*, 2008). The *E. coli* isolates were investigated for Extraintestinal Pathogenic *E. coli* related virulence genes (VGs) *kpsMII*, *papA*, *papC*, *iutA*, *sfaS*, *focG*, *afa* and *hlyD* (Jakobsen *et al.*, 2010b).

In 8 of the 13 (62%) RUTI patients (patient nos 1, 3, 4, 5, 6, 7, 10 and 12; 2–7 isolates per patient), the investigated characteristics of the isolates (PFGE type, phylogenetic group, antimicrobial resistance and VG profile) were similar among index and all recurrent isolates (Table 1, Fig. 1). Variation in solely the VG

profile was observed among isolates from one patient (patient no. 9, additional *kpsMII* VG in one isolate), and variation in solely the resistance profile was also observed among isolates from one patient only (patient no. 13, isolate no. 3 ciprofloxacin resistant). More widespread variation among isolates from the same patient was observed in three patients. In isolates from patient no. 8, all investigated characteristics varied among the two isolates, and in patient no. 11 strains varied except for phylogenetic grouping (both belonged to D). Among the three isolates recovered from patient no. 2, the first and second isolates (46 days between culturing) were similar regarding all the investigated characteristics, whereas the third isolate belonged to another PFGE type and phylogenetic group compared with the first two isolates (Table 1). Fig. 1 shows a PFGE gel with PFGE profiles from six patients; all but one patient showed an *E. coli* strain at recurrence(s) that was the same as the index strain. Most isolates belonged to phylogenetic lineages B2 ($n=14$, 34%) and D ($n=11$, 27%). This agrees with most other studies concerning phylogenetic groups in uropathogenic *E. coli* (Johnson *et al.*, 2005; Moreno *et al.*, 2008). All VGs except *focG* were detected among the isolates. In isolates from two patients, none of the investigated VGs was found. The most commonly found VGs were *kpsMII* (nine patients) and *iutA* (nine patients) (Table 1). The *E. coli* isolates from the first UTI episode in each of the 13 patients have been part of a cluster analysis considering correlation between VG profile and phenotypic antimicrobial-resistance profile (Jakobsen *et al.*, 2010b). Ciprofloxacin resistance was found in one isolate (patient no. 13). In this patient, from whom four episodes of UTI were observed (the four isolates had similar PFGE profiles), the isolated strain changed from ciprofloxacin susceptible (isolate nos 1 and 2) to ciprofloxacin resistant (isolate no. 3). This was connected with treatment

Table 1. Characteristics of *E. coli* isolates (*n*=41) from 13 patients with RUTI

Patient no.	Sex, age	Underlying disease	Isolate no.	Date of infection (culture)	PFGE type (clone)	Phylogenetic group	Antimicrobial resistance	VG(s)	Antimicrobial therapy*
1	F, 59	No	3	9 December 2005	A	Non-typable	TET	None	None
			57	3 February 2006	A	Non-typable	TET	None	Mecillinam
			117	20 March 2006	A	Non-typable	TET	None	None
2	F, 80	No	7	16 December 2005	B	Non-typable	TMP, SMZ, TET	<i>kpsMII</i>	None
			54	2 February 2006	B	Non-typable	TMP, SMZ, TET	<i>kpsMII</i>	Mecillinam
			108	7 March 2006	C	D	TMP, SMZ, AMP, TET	<i>kpsMII</i>	Mecillinam
3	M, 60	No	10	13 December 2005	D	B2	TMP, TET	<i>iutA, papC, papA, kpsMII, hlyD</i>	Mecillinam
			22	28 December 2005	D	B2	TMP, TET	<i>iutA, papC, papA, kpsMII, hlyD</i>	Mecillinam
			52	25 January 2006	D	B2	TMP, TET	<i>iutA, papC, papA, kpsMII, hlyD</i>	Mecillinam
4	F, 43	No	32	10 January 2006	E	B2	SMZ, AMP	<i>iutA, papC, papA, kpsMII</i>	Sulfamethizole, then nitrofurantoin
5	F, 65	No	51	24 January 2006	E	B2	SMZ, AMP	<i>iutA, papC, papA, kpsMII</i>	Nitrofurantoin
			33	5 January 2006	F	B1	TMP, SMZ, AMP	<i>iutA</i>	None
			103	2 March 2006	F	B1	TMP, SMZ, AMP	<i>iutA</i>	None
6	F, 70	Hypertension, asthma	40	11 January 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Nitrofurantoin
			50	23 January 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Mecillinam
			63	6 February 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Mecillinam
			91	20 February 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Nitrofurantoin
			104	6 March 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Nitrofurantoin
			112	17 March 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Sulfamethizole
			127	3 April 2006	G	Non-typable	TMP, SMZ, AMP, TET	<i>iutA, kpsMII</i>	Mecillinam
7	M, 62	No	56	30 January 2006	H	B2	AMP	<i>iutA, kpsMII, hlyD</i>	Sulfamethizole
			82	13 February 2006	H	B2	AMP	<i>iutA, kpsMII, hlyD</i>	Mecillinam
			92	22 February 2006	H	B2	AMP	<i>iutA, kpsMII, hlyD</i>	Mecillinam
			111	21 March 2006	H	B2	AMP	<i>iutA, kpsMII, hlyD</i>	Mecillinam
			125	5 April 2006	H	B2	AMP	<i>iutA, kpsMII, hlyD</i>	Mecillinam

Table 1. cont.

Patient no.	Sex, age	Underlying disease	Isolate no.	Date of infection (culture)	PFGE type (clone)	Phylogenetic group	Antimicrobial resistance	VG(s)	Antimicrobial therapy*
8	F, 66	Cystocele, incontinence	58	1 February 2006	I	B2	None	<i>papC, papA, kpsMII, sfaS, hlyD</i>	Trimethoprim
			126	30 March 2006	J	D	TMP, SMZ, AMP, TET	<i>iutA</i>	None
9	F, 76	Urine retention, recurrent urethral catheterization	61	6 February 2006	K	B2	TMP, SMZ, AMP, TET	<i>papC, papA, hlyD</i>	Sulfamethizole
			78	13 February 2006	K	B2	TMP, SMZ, AMP, TET	<i>papC, papA, hlyD</i>	None
			89	20 February 2006	K	B2	TMP, SMZ, AMP, TET	<i>papC, papA, kpsMII, hlyD</i>	Nitrofurantoin
10	F, 17	No	62	7 February 2006	L	D	TMP, SMZ, AMP, TET	<i>iutA, papC, papA, kpsMII</i>	Mecillinam
			84	14 February 2006	L	D	TMP, SMZ, AMP, TET	<i>iutA, papC, papA, kpsMII</i>	Nitrofurantoin
			102	10 March 2006	L	D	TMP, SMZ, AMP, TET	<i>iutA, papC, papA, kpsMII</i>	Nitrofurantoin
11	F, 31	No	68	1 February 2006	M	D	None	None	Sulfamethizole
			86	20 February 2006	N	D	TMP, SMZ, AMP, TET	<i>afa, iutA, papC, papA, hlyD</i>	None
12	F, 66	No	71	31 January 2006	O	Non-typable	None	None	Sulfamethizole
			81	13 February 2006	O	Non-typable	None	None	Sulfamethizole
13	M, 81	No	77	8 February 2006	P	D	TMP, SMZ, AMP	<i>iutA, kpsMII</i>	Amoxicillin
			87	20 February 2006	P	D	TMP, SMZ, AMP	<i>iutA, kpsMII</i>	Ciprofloxacin
			110	16 March 2006	P	D	CIP, TMP, SMZ, AMP	<i>iutA, kpsMII</i>	Amoxicillin, then mecillinam
			121	28 March 2006	P	D	TMP, SMZ, AMP	<i>iutA, kpsMII</i>	Mecillinam

AMP, Ampicillin; CIP, ciprofloxacin; F, female; M, male; SMZ, sulfamethizole; TET, tetracycline; TMP, trimethoprim.

*It is possible that the patients received antimicrobial therapy between the UTI episodes; however, this was not recorded.

Fig. 1. PFGE profiles from patient isolates nos 7 and 9–13 showing the index isolates (1) and isolates from recurrent episodes (.2–.5). MS is the molecular size standard (low range PFG marker; New England BioLabs) and IC the internal control. All patients except for patient no. 11 had *E. coli* isolates at recurrence(s) identical to the originally infecting (index) *E. coli* isolate (underlined).

of the second episode with ciprofloxacin. A new ciprofloxacin-susceptible isolate was found in the fourth UTI episode (isolate no. 4) after amoxicillin followed by mecillinam had been used for the third episode (Table 1).

This present study shows a high incidence of bacterial persistence during RUTI caused by *E. coli*. PFGE was one of the methods used to identify the strains and according to this, 77 % (10/13) of the patients in our study had unique clones of *E. coli* persisting during the RUTI episodes, i.e. the majority of UTIs in recurrent episodes were attributable to bacterial persistence with the primary infecting *E. coli* strain. The same percentage of RUTI caused by a relapse (77 %) was found in another study (Ejrnæs *et al.*, 2006). Taking into consideration all the investigated parameters, only 62 % (8/13) of the patients had similar clones of *E. coli* persisting during the RUTI course. However, two other patients had shifts in resistance to one antimicrobial substance and one VG only, respectively. It is possible that isolates from these two patients were indeed clones.

In conclusion, owing to the high degree of comparability regarding PFGE, phylotypes, virulence and antimicrobial resistance in 41 strains of infecting *E. coli* in this study, we propose that recurrent community-acquired UTIs in both women and men are most often infections caused by an earlier infecting strain. The finding of persisting *E. coli* clones not only supports the theory of a rectal or vaginal reservoir but also supports the recent theory of an intracellular bladder reservoir for uropathogenic *E. coli* (Rosen *et al.*, 2007).

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