Uropathogens from diabetic patients in Libya: virulence factors and phylogenetic groups of *Escherichia coli* isolates

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Urinary tract infections (UTIs) in patients with diabetes mellitus (DM) are reported mainly from developed countries. In addition to this underreporting from developing countries, there is a lack of information pertaining to the virulence factors (VFs) and phylogenetic grouping of uropathogenic *Escherichia coli* (UPEC) from DM and non-DM patients in developing countries. Between July 2005 and June 2006, urine specimens were collected from 135 DM and 164 non-DM patients, all with clinically diagnosed UTIs, attending Elkhadra Hospital and the Diabetic Center in Tripoli, Libya. Specimens were examined for different uropathogens using standard microbiological procedures. Isolated uropathogens were tested for their susceptibility to antimicrobial agents by a disc diffusion method. In addition, UPEC was grouped phylogenetically by PCR and subsequently tested for 19 VFs. Uropathogens were isolated from 77 (57 %) of the DM group and from 110 (67 %) of the non-DM group ($P > 0.05$). *E. coli* was isolated from 18 (13 %) and 29 (18 %), *Klebsiella* species from 18 (13 %) and 23 (14 %), and *Staphylococcus aureus* from 12 (9 %) and 12 (7 %) of the DM and non-DM groups, respectively ($P > 0.05$). Age, gender, education level and marital status had no significant influence on the isolation rates of different organisms from the DM group compared with the non-DM group. With very few exceptions, no differences were observed in the antimicrobial resistance profiles of uropathogens from the DM and non-DM patients. In addition, UPEC from the DM patients was significantly less virulent and was associated with phylogenetic group A, whilst UPEC from the non-DM patients was significantly more virulent and was associated with group D. The results of our surveillance of UTI infections in DM patients agree, in general, with observations reported previously from several developed countries.

Received 7 October 2008

Accepted 3 May 2009

INTRODUCTION

A number of investigators have observed that both common and rare infections are reported more frequently among patients with diabetes mellitus (DM) than in the general population, although this is not universally accepted (Calvet & Yoshikawa, 2001; Hu *et al.*, 2004; Pozzilli & Leslie, 1994). It has been suggested by various

epidemiological studies that bacteriuria and urinary tract infections (UTIs) are more common in women with DM than in non-DM women (Patterson & Andriole, 1995).

Several factors have been implicated in the higher prevalence of asymptomatic bacteriuria and incidence of UTIs in patients with DM compared with patients without DM. These factors include differences in host responses between DM and non-DM patients, a difference in the infecting bacterium itself, the presence of glucosuria and impairment of granulocyte function (Geerlings, 2008). However, several investigators found no differences in the above-mentioned

Abbreviations: DM, diabetes mellitus; MDR, multidrug resistance; OR, odds ratio; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; VF, virulence factor.

factors in DM compared with non-DM patients (Balasoiu *et al.*, 1997; Geerlings *et al.*, 2000a, b, 2001).

Escherichia coli is the most common causative agent of UTIs in both DM and non-DM patients (Bonadio *et al.*, 2006). Other organisms reported include members of the family *Enterobacteriaceae* (i.e. *Proteus*, *Klebsiella*, *Enterobacter* and *Citrobacter* species), *Pseudomonas* species, *Enterococcus* species, streptococci, staphylococci and *Candida albicans* (Ludwig, 2000).

Uropathogenic *E. coli* (UPEC) possesses a variety of pathogenicity determinants that make colonization of the urinary tract possible. These determinants include fimbrial (P, S/F1C and type 1 fimbriae) and non-fimbrial adhesins that mediate bacterial adherence to epithelial cells, siderophores (iron-acquisition systems), secreted toxins (haemolysin and cytotoxic necrotizing factor 1) and capsule-forming polysaccharides for immune evasion (Bower *et al.*, 2005; Tseng *et al.*, 2001; Wiselka, 1996).

Phylogenetic analysis classifies *E. coli* strains into four main groups (A, B1, B2 and D). Groups B2 and D are mainly associated with *E. coli* strains causing extraintestinal infections, whilst groups A and B1 are associated with commensal strains (Picard *et al.*, 1999).

Worldwide, there is great concern due to the high rates of resistance to antimicrobials used in the treatment of infections caused by *E. coli*, particularly in developing countries. In industrial countries, ciprofloxacin and other fluoroquinolones are used as the drugs of choice in empirical treatment of uncomplicated UTIs by uropathogens with resistance rates to trimethoprim-sulphamethoxazole exceeding 10% (Warren *et al.*, 1999). In developing countries, there is a dearth of information on the antimicrobial susceptibility of *E. coli* from DM patients with UTIs to the newly available drugs including the fluoroquinolones and third-generation cephalosporins.

Studies investigating UTIs in patients with DM have primarily examined the situation in developed countries. In addition to the lack of adequate surveillance information, there has been a lack of isolate characterization [phylogenetics, antimicrobial susceptibility testing, virulence factors (VFs)] of UPEC from DM and non-DM patients in developing countries. The present work was carried out to determine the causative organisms of UTIs in DM patients in Tripoli, Libya, compared with non-DM patients, their susceptibility to the commonly used antimicrobial agents, their phylogenetic grouping and the VFs of UPEC isolated from these patients. The influence of age, gender, marital status and education level on the species and frequency of isolation of organisms from DM compared with non-DM patients was also investigated.

METHODS

Patients. In total, 299 patients (135 with DM and 164 non-DM) with clinically diagnosed UTIs presenting at Elkhadra Hospital or the

Diabetic Center in Tripoli were included. Patients with a UTI were diagnosed by having one or more of the following symptoms: dysuria, frequency, urgency, suprapubic discomfort or flank pain. The age of the DM group ranged from 10 to 78 years (mean 48.4 years) and that of the non-DM group from 3 to 83 years (mean 35.2 years). Information on gender, marital status and education level were obtained for each patient. The study was carried out between July 2005 and June 2006. All patients were informed of the purpose of the study and their consent or that of their care provider was obtained before urine samples were collected.

Specimen collection. Urine specimens were obtained from all patients included in the study with the assistance of a medical doctor or senior nurse. Female patients were asked to clean their labia with water and soap before urine collection. Approximately 20 ml mid-stream urine was collected from each patient in a sterile container, placed on an ice bag and processed in the laboratory within 2 h of collection. None of the patients admitted to consuming antibiotics during the 2 weeks prior to urine sample collection.

Isolation and identification of uropathogens. Using calibrated loops (10^{-3} ml, Last Loop Sp; Italiana), urine specimens were plated on MacConkey and blood agar plates. After 18–24 h incubation at 37 °C, the number of c.f.u. ml⁻¹ was counted and urine samples giving $\geq 10\,000$ c.f.u. ml⁻¹ were considered significant (Wilson & Gaido, 2004). Isolated colonies from significant plates were identified using standard bacteriological procedures (Collee *et al.*, 1989) and the biochemical systems API 20E and API 20NE (bioMérieux). Urine samples were also plated onto Sabouraud agar for detection of *C. albicans*: plates were incubated at 37 °C for 24–48 h and suspected colonies of *C. albicans* were confirmed by a germ-tube test (Koneman *et al.*, 1997).

Antimicrobial susceptibility testing. A disc diffusion method (Jorgeensen & Turnidge, 2003) was used to determine the susceptibility of *E. coli*, *Klebsiella* species and *Staphylococcus aureus* isolates to different antimicrobial agents. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as control organisms. In addition, *E. coli* isolates were tested for extended-spectrum β -lactamase production according to published guidelines (CLSI, 2005).

Detection of *E. coli* virulence determinants. Nineteen virulence determinants from *E. coli* isolates in this study were detected by PCR. These targets were part of a large set of virulence genes described by Johnson & Stell (2000). The conditions used for each PCR assay were essentially as described previously (Johnson & Stell, 2000); however, rather than using all of the primer pairs described by these authors, we used the following: pool 1: *PAI* (925 bp), *papA* (717 bp), *fimH* (508 bp), *papEF* (326 bp), *kpsMT III* (392 bp) and *ibeA* (171 bp); pool 2: *fyuA* (787 bp), *bmaE* (507 bp), *sfal/focD* (410 bp) and *iutA* (302 bp); pool 3: *hlyA* (1177 bp), *rfc* (788 bp), *nfaE* (539 bp), *kpsMT II* (272 bp) and *papC* (205 bp); pool 4: *cvaC* (657 bp), *focG* (364 bp) and *traT* (290 bp); pool 5: *afal/draBC* (594 bp). The primer pairs for these 19 virulence genes and the amplification conditions have been described elsewhere (Huang *et al.*, 1995; Johnson & Brown, 1998; Johnson & Stell, 2000; Le Bouguenec *et al.*, 1992; Schubert *et al.*, 1998; Yamamoto *et al.*, 1995). Boiled whole-cell lysates (400 µl) from each isolate were used as DNA template and amplification was carried out in a 25 µl reaction mixture containing 4 mM MgCl₂, 600 µM each dNTP, 10 µl 5 × Green GoTaq Flexi buffer (Promega), 2.5 U GoTaq Flexi DNA polymerase (Promega), 0.5 µM each primer and 5 µl whole-cell lysate. Reactions were performed in a GeneAmp PCR System 9700 (Applied Biosystems).

DNA sequencing was used to confirm the identity of the amplified PCR products and to establish positive controls. Initially, the nucleotide sequence of an amplified PCR product of each virulence

gene, representing a single isolate, was determined using the Sanger dideoxy chain-termination method. The DNA sequence was submitted to the BLAST nucleotide resource at the National Center for Biotechnology Information for identification. Once a PCR product for an individual virulence determinant was confirmed, the DNA from this isolate was used as a positive control for all subsequent PCRs. A reaction mixture lacking a DNA template was used as a negative control in each PCR assay.

Detection of *E. coli* haemolysin production. Isolates were plated on sheep-blood agar and incubated at 37 °C for 24 h. Isolates that produced a clear zone of haemolysis around colonies were considered to be positive for haemolysin production.

Phylogenetic grouping of *E. coli* isolates. Phylogenetic grouping (A, B1, B2 or D) of the *E. coli* isolates was performed using a multiplex PCR method as described previously (Clermont *et al.*, 2000).

Statistical analysis. For statistical analysis, Epi Info 2000 software (Centers for Disease Control and Prevention) was employed. *P* values were calculated using a χ^2 test. *P* < 0.05 was considered to be statistically significant.

RESULTS

Uropathogens were isolated from 77 (57%) of the DM group and from 110 (67%) of the non-DM group. The difference in the isolation rates of uropathogens from the two groups of patients was not statistically significant (*P* > 0.05). Of the isolated uropathogens, *E. coli* was isolated from 18 (13%) and 29 (18%), *Klebsiella* species from 18 (13%) and 23 (14%), and *S. aureus* from 12 (9%) and 12 (7%) of the patients in the DM and non-DM groups, respectively. These differences were not statistically significant (*P* > 0.05). Table 1 shows the organisms detected in urine samples examined from the two groups. It should be noted that two biochemically different *E. coli* strains were isolated from one DM patient.

No significant differences (*P* > 0.05) were observed in the isolation rates of uropathogens from patients ≤ 40 or > 40 years of age, females or males, single or married subjects, and those with or without a university degree in the two groups. Table 2 shows the distribution of *E. coli*, *Klebsiella* species and *S. aureus* isolated from DM and non-DM patients according to age, gender, education level and marital status. Furthermore, no significant differences were found in the isolation rates of uropathogens from DM patients using oral medications when compared with those taking insulin (*P* > 0.05).

Table 3 shows the resistance patterns to antimicrobial agents of *E. coli*, *Klebsiella* species and *S. aureus* isolated from DM and non-DM patients. Although *E. coli* from DM patients showed the higher resistance rates to most of the drugs tested compared with non-DM patients, the differences were not statistically significant (*P* > 0.05). In contrast, *Klebsiella* species from non-DM patients showed higher resistance rates to most of the antimicrobial agents compared with isolates from DM patients. *Klebsiella* species from the non-DM group were significantly more resistant than isolates from the DM group to amoxicillin-clavulanic acid [*P* < 0.002, odds ratio (OR) = 13.13]. *S. aureus* isolates from the non-DM group were resistant to most of the antimicrobial agents tested, including meti-cillin, compared with isolates from the DM group; however, the differences were not statistically significant (*P* > 0.05). Of the *E. coli*, *Klebsiella* species and *S. aureus* isolates in this investigation from both DM and non-DM patients, ≥ 40, ≥ 65 and ≥ 29% showed multiple drug resistance profiles (resistance to three or more antibiotics), respectively. Of 48 *E. coli* isolates examined, extended-spectrum β-lactamases were detected in two isolates (4%; one from a DM patient and one from a non-DM patient).

Of the 19 VFs examined, 14 were detected, ranging from none to six VFs (mean three) in *E. coli* isolates from DM

Table 1. Uropathogens isolated from DM and non-DM patients with UTIs

Organism	No. (%) positive in:		
	DM patients with UTIs (n=135)	Non-DM patients with UTIs (n=164)	Total (n=299)
<i>E. coli</i>	18 (13)	29 (18)	47 (16)
<i>Klebsiella</i> species	18 (13)	23 (14)	41 (14)
<i>Proteus</i> species	2 (1.5)	9 (5.5)	11 (4)
<i>Enterobacter</i> species	7 (5)	7 (4)	14 (5)
<i>Citrobacter</i> species	2 (1.5)	4 (2)	6 (2)
<i>Serratia</i> species	0 (0.0)	3 (2)	3 (1)
<i>Pseudomonas</i> species	6 (4)	7 (4)	13 (4)
<i>Acinetobacter</i> species	1 (1)	5 (3)	6 (2)
<i>S. aureus</i>	12 (9)	12 (7)	24 (8)
<i>Staphylococcus saprophyticus</i>	1 (1)	2 (1)	3 (1)
<i>Candida albicans</i>	10 (7)	9 (5.5)	19 (6)
Total	77 (57)	110 (67)	187 (62.5)
No organism identified	58 (43)	54 (33)	112 (37.5)

Table 2. Distribution of *E. coli*, *Klebsiella* species and *S. aureus* isolated from DM and non-DM patients with UTIs according to age, gender, education level and marital status

Age, gender, education level and marital status had no statistically significant influence ($P > 0.05$) on the isolation of *E. coli*, *Klebsiella* species or *S. aureus* from DM and non-DM patients with UTIs.

Characteristic	No. of DM patients	No. (%) of isolates of:			No. of non-DM patients	No. (%) of isolates of:		
		<i>E. coli</i>	<i>Klebsiella</i> species	<i>S. aureus</i>		<i>E. coli</i>	<i>Klebsiella</i> species	<i>S. aureus</i>
Age (years)								
≤40	34	5 (15)	6 (18)	3 (9)	119	21 (18)	16 (13.5)	8 (7)
>40	101	13 (13)	12 (12)	9 (9)	45	8 (18)	7 (16)	4 (9)
Gender								
Female	97	12 (12)	13 (13)	11 (11)	83	18 (22)	8 (10)	7 (8)
Male	38	6 (16)	5 (13)	1 (3)	81	11 (14)	15 (18.5)	5 (6)
Education level								
No university	120	12 (10)	12 (10)	11 (9)	110	19 (17)	13 (12)	7 (6)
University	15	6 (40)	6 (40)	1 (7)	54	10 (18.5)	10 (18.5)	5 (9)
Marital status								
Single	83	11 (13)	12 (14.5)	7 (8)	71	15 (21)	13 (18)	7 (10)
Married	52	7 (13.5)	6 (11.5)	5 (10)	93	14 (15)	10 (11)	5 (5)

patients and from one to nine VFs (mean 4.4) in *E. coli* isolates from non-DM patients. Of the total *E. coli* isolates studied, three or more VFs (multivirulent isolates) were found in >56 % (multivirulent isolates). Detection of multivirulent genes was statistically significant in *E. coli* from non-DM patients (25/29, 86 %) compared with *E. coli* from DM patients (11/19, 58 %) ($P < 0.03$, OR=4.55).

Only the *fimH* (type 1 fimbriae) and *traT* (serum resistance) genes were statistically significantly more common in *E. coli* from non-DM than from DM patients (93 vs 63 % and 79 vs 21 %; $P < 0.01$ OR=7.88, and $P < 0.00007$ OR=14.38, respectively) (Table 4). Haemolysin production on sheep blood agar was detected in all *hlyA*-positive *E. coli* isolates.

Table 3. Antimicrobial sensitivity patterns of *E. coli*, *Klebsiella* species and *S. aureus* isolated from DM and non-DM patients with UTIs

Antimicrobial agent	No. (%) of drug-resistant isolates from:					
	DM patients with UTIs			Non-DM patients with UTIs		
	<i>E. coli</i> (n=19)*	<i>Klebsiella</i> species (n=18)	<i>S. aureus</i> (n=12)	<i>E. coli</i> (n=29)	<i>Klebsiella</i> species (n=23)	<i>S. aureus</i> (n=12)
Ampicillin	12 (63)	18 (100)	8 (67)	17 (59)	23 (100)	7 (58)
Amoxicillin + clavulanic acid	8 (42)	8 (44)	1 (8)	9 (31)	21 (91)	4 (33)
Cephalothin	9 (47)	10 (56)	ND	19 (66)	17 (74)	ND
Cefotaxime	1 (5)	4 (22)	1 (8)	1 (3.5)	7 (30)	4 (33)
Chloramphenicol	2 (11)	6 (33)	2 (17)	6 (21)	4 (17)	1 (8)
Gentamicin	2 (11)	6 (33)	0 (0.0)	3 (10)	9 (39)	4 (33)
Nalidixic acid	6 (32)	8 (44)	ND	8 (28)	10 (44)	ND
Ciprofloxacin	6 (32)	4 (22)	0 (0.0)	4 (14)	8 (35)	1 (8)
Streptomycin	6 (32)	ND	ND	14 (48)	ND	ND
Trimethoprim-sulphamethoxazole	6 (32)	3 (17)	0 (0.0)	7 (24)	2 (9)	0 (0.0)
Tetracycline	ND	ND	5 (42)	ND	ND	7 (58)
Meticillin	ND	ND	1 (8)	ND	ND	4 (33)
Fusidic acid	ND	ND	3 (25)	ND	ND	4 (33)
Vancomycin	ND	ND	0 (0.0)	ND	ND	0 (0.0)

ND, Not done.

*Two biochemically different *E. coli* strains were isolated from one diabetic patient.

Table 4. Distribution of VFs and phylogeny groups among UPEC isolated from DM and non-DM patients and in relation to ciprofloxacin resistance

VF and phylogeny group	No. of associated VFs (%)			
	Diabetics (n=19)	Non-diabetics (n=29)	Ciprofloxacin-susceptible (n=38)	Ciprofloxacin-resistant (n=10)
<i>papA</i> (P fimbriae)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>papC</i> (P fimbriae)	1 (5)	3 (10)	4 (11)	0 (0.0)
<i>papEF</i> (P fimbriae)	1 (5)	3 (10)	4 (11)	0 (0.0)
<i>sfa/focD</i> (Dr family adhesions)	2 (11)	8 (28)	10 (26)	0 (0.0)
<i>focG</i> (F1C fimbriae)	1 (5)	7 (24)	8 (21)	0 (0.0)
<i>afa/draBC</i> (Dr family adhesions)	0 (0.0)	4 (14)	4 (11)	0 (0.0)
<i>bmaE</i> (M fimbriae)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>nfaE</i> (non-fimbrial adhesin-1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>fimH</i> (type 1 fimbriae)	12 (63)	27 (93)*	32 (84)†	4 (40)
<i>hlyA</i> (haemolysin)	4 (21)	7 (24)	11 (29)‡	0 (0.0)
<i>fyuA</i> (yersiniabactin)	12 (63)	11 (38)	19 (50)	4 (40)
<i>iutA</i> (aerobactin)	13 (68)	21 (72)	28 (74)§	4 (40)
<i>kpsMT II</i> (capsule synthesis group II)	7 (37)	11 (38)	18 (47)	0 (0.0)
<i>kpsMT III</i> (capsule synthesis group III)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>rfa</i> (O4 LPS)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>ibeA</i> (invasion)	0 (0.0)	1 (4)	1 (3)	0 (0.0)
<i>cvaC</i> (colicin V)	0 (0.0)	1 (4)	1 (3)	0 (0.0)
<i>traT</i> (serum resistance)	4 (21)	23 (79)¶	23 (61)	4 (40)
PAI (pathogenicity-associated island)	0 (0.0)	2 (7)	1 (3)	1 (10)
Multivirulent (≥ three genes)	11 (58)	25 (86)#	34 (90)**	2 (20)
Phylogenetic group				
A	14 (74)††	4 (14)	10 (26)	8 (80)‡‡
B1	0 (0.0)	1 (3.4)	1 (3)	0 (0.0)
B2	3 (16)	8 (28)	10 (26)	1 (10)
D	2 (11)	16 (55)§§	17 (45)	1 (10)

**fimH* is significantly associated with *E. coli* from non-DM patients compared with *E. coli* from DM patients ($P < 0.01$, OR=7.88).

†*fimH* is significantly associated with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P < 0.005$, OR=8.00).

‡*hlyA* is strongly associated with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P = 0.05$, OR=undefined).

§*iutA* is significantly associated with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P < 0.05$, OR=4.20).

||*kpsMT II* is significantly associated with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P < 0.006$, OR=undefined).

¶*traT* is significantly associated with *E. coli* from non-DM patients compared with *E. coli* from DM patients ($P < 0.00007$, OR=14.38).

#Multivirulence is significantly associated with *E. coli* from non-DM patients compared with *E. coli* from DM patients ($P < 0.03$, OR=4.55).

**Multivirulence is significantly associated with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P < 0.000007$, OR=34.00).

††*E. coli* from diabetics is significantly associated with phylogeny group A compared with *E. coli* from non-DM patients ($P < 0.00003$, OR=17.50).

‡‡Ciprofloxacin-resistant *E. coli* is significantly associated with phylogeny group A compared with ciprofloxacin-susceptible *E. coli* ($P < 0.002$, OR=11.20).

§§*E. coli* from non-DM patients is significantly associated with phylogeny group D compared with *E. coli* from DM patients ($P < 0.002$, OR=10.46).

||||Ciprofloxacin-susceptible *E. coli* is significantly associated with phylogeny group D compared with ciprofloxacin-resistant *E. coli* ($P < 0.05$, OR=7.29).

Multivirulent genes were found to be significantly more common in ciprofloxacin-susceptible UPEC (34/38, 90 %) than among ciprofloxacin-resistant UPEC (2/10, 20 %) ($P < 0.000007$, OR=34.00). In addition, *fimH*, *iutA* (aerobactin) and *kpsMT II* (capsule synthesis group II) were found significantly more often in ciprofloxacin-susceptible than in ciprofloxacin-resistant *E. coli* isolates (84 vs 40 %, 74 vs 40 % and 47 vs 0.0 %; $P < 0.005$ OR=8.00, $P < 0.05$ OR=4.20 and $P < 0.006$ OR=undefined, respectively). The *hlyA* (haemolysin) gene was found to be strongly associated

with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P = 0.05$, OR=undefined).

The distribution of phylogenetic groups among UPEC isolated from DM and non-DM patients is shown in Table 4. Significantly more *E. coli* from the DM patients (14/19, 74 %) than from the non-DM patient group (4/29, 14 %) belonged to group A ($P < 0.00003$, OR=17.50). However, significantly more *E. coli* from the non-DM group (16/29, 55 %) than from the DM group (2/19, 11 %) with ciprofloxacin-susceptible compared with ciprofloxacin-resistant *E. coli* ($P = 0.05$, OR=undefined).

belonged to group D ($P < 0.002$, $OR = 10.46$). Although more *E. coli* from the non-DM group (8/29, 28 %) than from the DM group (3/19, 16 %) belonged to phylogenetic group B2, the difference was not significant statistically ($P > 0.05$). In addition, phylogenetic group A was statistically significantly associated with ciprofloxacin-resistant UPEC (8/10, 80 %) compared with ciprofloxacin-susceptible UPEC (10/38, 26 %; $P < 0.002$, $OR = 11.20$). Furthermore, phylogenetic group D was statistically significantly associated with ciprofloxacin-susceptible UPEC (17/38, 45 %) compared with ciprofloxacin-resistant UPEC (1/10, 10 %; $P < 0.05$, $OR = 7.29$). The distribution of phylogenetic groups in relation to ciprofloxacin resistance among UPEC isolates is shown in Table 4.

The presence of multivirulent genes (three or more VFs) was found to be statistically significant in group B2 (10/11, 91 %) and group D (16/18, 89 %) compared with group A (8/18, 44 %) ($P < 0.02$, $OR = 12.50$ and $P < 0.005$, $OR = 10.00$, respectively).

Table 5 shows the distribution of VFs and phylogeny groups among UPEC according to the multidrug resistance (MDR, resistance to three or more antimicrobials) phenotype. Of the six most common detected VFs, only *fimH* was found to be statistically significantly associated with non-MDR UPEC when compared with MDR UPEC isolates. In addition, MDR UPEC isolates were significantly associated with phylogenetic group B2 when compared with non-MDR UPEC isolates.

DISCUSSION

This investigation was carried out in a developing country, although the findings confirm what has been reported previously from several developed countries.

E. coli was the most commonly recovered uropathogen in DM and non-DM patients with UTIs in our study. However, we found that there was a trend towards a lower proportion of UTIs caused by this organism in DM compared with non-DM patients (13 vs 18 %, respectively). Other investigators have reported similar findings (Hansen *et al.*, 1998; Lye *et al.*, 1992). In Italy, Bonadio *et al.* (1999) found that the most frequent causative agents of UTIs in DM and non-DM patients were *E. coli*, *Proteus* species, *Pseudomonas* species and *Enterococcus* species. In addition to *E. coli*, we detected *Klebsiella* species and *S. aureus* as the most common uropathogens in our study population. Although our findings are similar to those of Bonadio *et al.* (1999) as far as *E. coli* is concerned, the differences in the detection of other uropathogens might be the result of the different populations studied, as most of their DM patients had asymptomatic bacteriuria and had undergone bladder catheterization more frequently than the non-DM group. Our DM and non-DM patients were clinically diagnosed with UTIs and none had undergone bladder catheterization.

DM is a common predisposing factor for UTIs caused by fungi, particularly *Candida* species (Joshi *et al.*, 1999). Similar to our observations with *Klebsiella* species, *C. albicans* was detected at approximately the same proportion in the DM and non-DM groups (7 vs 5.5 %, respectively).

Boyko *et al.* (2002) examined the risk of UTIs among post-menopausal women with DM. They found higher UTI odds were associated mainly with use of oral hypoglycaemic agents and insulin, but not with DM treated with lifestyle changes or left untreated. We found no significant differences in the isolation rates of uropathogens from DM

Table 5. Distribution of bacterial VFs and phylogeny groups among UPEC according to MDR phenotype

	No. (%) of isolates with characteristic		P value
	Non-MDR* (n=22)	MDR† (n=26)	
VF			
<i>fimH</i>	21 (95)	18 (69)	$P < 0.03$, $OR = 9.33$
<i>traT</i>	10 (45)	17 (65)	NS
<i>fyuA</i>	9 (41)	14 (54)	NS
<i>kspMT II</i>	8 (36)	10 (38)	NS
<i>iutA</i>	18 (82)	16 (62)	NS
Haemolysin	5 (23)	6 (23)	NS
Multivirulent ($\geq$ three VFs)	18 (82)	18 (69)	NS
Phylogenetic group			
A	9 (41)	9 (35)	NS
B1	—	1 (4)	NS
B2	2 (9)	9 (35)	$P < 0.04$, $OR = 5.29$
D	11 (50)	7 (27)	NS

NS, Not significant ($P > 0.05$).

*Non-MDR, resistant to two or fewer antimicrobial agents.

†MDR, resistance to three or more antimicrobial agents.

patients using oral medications when compared with DM patients taking insulin.

Antimicrobial resistance among uropathogens causing community- and hospital-acquired UTIs is increasing (Bonadio *et al.*, 2001). Few data are available on the role of DM itself as a risk factor for the development of antimicrobial resistance of the uropathogens. Recently, a study in Italy reported that the resistance of uropathogens to antibiotics was similar in patients with and without DM (Bonadio *et al.*, 2001). With few exceptions, we found no differences in the resistance profiles of *E. coli*, *Klebsiella* species or *S. aureus*, regardless of whether they were isolated from DM or non-DM patients with UTIs. Our findings and those of the investigators reported above suggest that DM per se is not associated with an increase in antimicrobial resistance. However, all classes of isolated uropathogens in the present study, regardless of their source, showed high rates of multiple antimicrobial resistance, particularly to some of the drugs commonly used for treatment of UTIs, and more than 20 % of our *E. coli* and *Klebsiella* species isolates were resistant to ciprofloxacin.

Many VFs have been reported in UPEC and are frequently associated with UTIs. Studies have shown that hosts with predisposing factors, such as DM, can acquire UTIs caused by less virulent *E. coli* strains (Johnson, 1991; Tseng *et al.*, 2002). In the present study, we have reported similar findings, with multivirulent UPEC isolates being detected significantly more in non-DM than in DM patients. It is worth mentioning that several of the VFs detected in the present study, such as *fyuA*, *kpsMT II* and *traT*, have not been reported previously in *E. coli* isolates from DM patients with UTIs. Johnson & Stell (2000) reported that *fyuA*, *kpsMT II*, *traT* and *fimH* were prevalent among *E. coli* strains isolated from both compromised (with an upper urinary tract abnormality) and non-compromised hosts. Of the 19 VFs examined here, we found only *fimH* (type 1 fimbriae) and *traT* (serum resistance) significantly associated with UPEC isolated from non-DM patients compared with UPEC from DM patients. On the other hand, no significant differences were found in the prevalence of *fyuA* (yersinia-bactin) and *kpsMT II* (capsule synthesis group II) among UPEC from DM and non-DM patients.

Previous studies have shown that aerobactin-positive *E. coli* strains are more common in isolates from patients with acute pyelonephritis than in faecal *E. coli* isolates from healthy individuals (Jacobson *et al.*, 1988). Other investigators have reported similar findings with *E. coli* strains from bacteraemic patients with and without DM compared with faecal strains (Brauner *et al.*, 1988). Although we did not compare our *E. coli* isolates with faecal strains, the virulence gene *iutA* (aerobactin) was detected at almost equal rates among *E. coli* isolates from DM and non-DM patients (68 vs 72 %).

Several investigators have reported that most VF-containing UPEC isolates cluster mainly in phylogeny group B2 or

are shared between groups B2 and D (Boyd & Hartl, 1998; Picard *et al.*, 1999). In the present study, VFs clustered approximately equally within groups B2 and D significantly more than in group A. However, it should be mentioned that some VFs were found at a higher proportion in group A than in group B2 or D. For example *fyu* was detected in 67 % of phylogeny group A, in 36 % of group B2 and in 39 % of group D (data not shown). Other investigators have reported similar observations (Johnson *et al.*, 2001). Our finding of a significant association of *E. coli* from DM patients with group A compared with *E. coli* from non-DM patients, and the significant association of *E. coli* from non-DM patients with group D compared with *E. coli* from DM patients, strongly support the notion that UTIs in DM patients are caused by less virulent UPEC than in non-DM patients.

Several recent studies have shown that resistance of UPEC to nalidixic acid and ciprofloxacin and to multiple drugs may be associated with decreased expression or loss of some VFs (e.g. type 1 fimbriae, P fimbriae and haemolysin) (Drews *et al.*, 2005; Johnson *et al.*, 2004, 2005; Vila *et al.*, 2002). We found that ciprofloxacin-susceptible UPEC was significantly more associated with VFs than ciprofloxacin-resistant UPEC. Also, of the several predominant VFs, only *fimH* was found to be significantly more common among non-MDR UPEC than among MDR UPEC isolates.

A number of studies (Johnson *et al.*, 2002, 2003, 2005) have reported a significant association of ciprofloxacin-resistant extraintestinal *E. coli* with phylogeny group A and ciprofloxacin-susceptibility with group B2. In agreement with their findings, we observed a significant association of ciprofloxacin-resistant UPEC with phylogeny group A. However, we found a significant association of group D with ciprofloxacin-susceptible UPEC isolates. Given the association with group D and virulence, this is probably not unexpected. In addition, MDR UPEC isolates were significantly associated with phylogeny group B2 when compared with non-MDR UPEC isolates. Johnson *et al.* (2004) examined 152 *E. coli* isolates (70 % from urine specimens), 50 % of which were MDR. They found that the MDR isolates possessed significantly lower prevalences of most of the screened VFs, including *kpsM II*, *hlyD* and *fimH*. Furthermore, they reported MDR and susceptible isolates as being significantly associated with phylogeny groups D and B2, respectively. Contrary to their findings, we found a significant association of MDR with group B2. It is not easy to elaborate on these conflicting findings; however, possible explanations include: (i) our UPEC isolates were from DM and non-DM patients whilst none of the isolates of Johnson *et al.* (2004) were from DM patients; (ii) we tested nine antimicrobials whilst they tested only five; (iii) our isolates were obtained from a population in a developing country where antimicrobials are easily available over the counter, with high rates of MDR, contrary to the situation in developed countries; and (iv) our UPEC isolates may have originated from ancestors that are different to those of Johnson *et al.* (2004).

Symptomatic bacteriuria in DM patients is serious and warrants proper clinical attention for both diagnosis and treatment. Paradoxically, its eradication appears to be more difficult in patients with DM (and less virulent *E. coli* strains) than in non-DM patients (with more virulent UPEC) (Andriole, 2002; Bonadio *et al.*, 1999). Although our findings indicate that *E. coli*, *Klebsiella* species and *S. aureus* are the most common uropathogens, urine cultures should be performed before and after therapy. The high rates of MDR among uropathogens observed in our study will no doubt limit the choice of drugs available for clinicians involved in the treatment and management of UTIs in DM patients in our region. Ciprofloxacin and cefotaxime were observed to be the most appropriate agents in the population studied. However, treatment should be tailored to local resistance patterns and the most likely causative agent in the individual patient (Stapleton, 2002). Because of differences in social, economic, health and environmental conditions between developed and developing countries, more studies are needed from developing countries to address the issues of treatment and management of UTIs in DM patients, using more defined populations of individuals with DM. Furthermore, studies are also needed on the prevalence of VFs and phylogenetic groups in UPEC from DM patients in developing areas. These studies will be important in understanding the role of these factors in causing UTIs in both DM and non-DM patients, which in turn may lead to the development of universal vaccines to prevent such infections.

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