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A study of uropathogenic *Escherichia coli* from men in regional NSW (Australia) with special emphasis on antibiotic resistance

Abstract

Background: *Escherichia coli* is an important uropathogen, responsible for most urinary tract infections (UTIs). Most studies on *E. coli* UTI pathogenesis have been in females, with limited studies on urinary isolates from men. Understanding UTI epidemiology in men is important in UTI management in this gender, including formulation of empirical treatment guidelines.

Methods: We studied the distribution of 310 uropathogenic *E. coli* isolates from men by uro-clinical syndrome, hospitalisation status, age group, and antibiotic susceptibility.

Results: Most of the *E. coli* isolates were from the outpatient setting (189, 61%), with the various age groups generally evenly distributed. In the inpatient setting, the 0-10 years age group was not represented, and the remaining 3 age groups were evenly represented (22-27%). Most of the isolates (74%) were from cystitis cases. For pyelonephritis isolates, the majority (65%) were confined to the 21-40 years age group, and the least in the 11-20 years age group (12%). Antibiotic (and multidrug) resistance was higher in pyelonephritis vs. cystitis isolates (73% vs. 58% ; P = 0.023, for multidrug resistance). Overall, antibiotic resistance differed significantly by age; highest in the 21-40 years age range (69%), and lowest in the 0-10 years group (39%). Likewise, ESBL production was highest in the 21-40 years age range (10%), and lowest in the 0-10 years age (0%).

Conclusion: We documented a significant difference in the distribution of antibiotic resistance by age group amongst UPEC isolates from men in regional NSW, Australia, in the 0-60 years age group.

Keywords: urinary tract infection, *Escherichia coli*, antibiotic resistance

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Introduction

Urinary tract infections (UTI) are responsible for considerable morbidity and associated healthcare costs worldwide.[1] In the United States of America (US), over 100 000 UTI associated hospital admissions occur each year,[2] resulting in significant consumption of health resources. More UTI episodes occur in women than men, with over 50% of all women expected to have at least one episode in their lifetime.[3] In most cases of UTI, *Escherichia coli* is the causative pathogen, hence most studies on UTI pathogenesis have targeted bacterial characteristics of this organism.

Effective management of UTI has become challenging over the years partly due to increasing rates of resistance to the commonly used antibiotics worldwide, especially in the developing world.[4] Most European countries have also recorded a significant rise in the rates of antibiotic resistance amongst urinary pathogens, especially for uropathogenic *E. coli* (UPEC).[5] In Australia, such rates are still low compared with many other countries, but they are also increasing.

Although there is much data in literature on antibiotic resistance in UPEC, most of it is derived from women, especially the reproductive age cohort, with very limited studies on bacterial isolates from men. Consequently, most treatment guidelines for UTIs (cystitis and pyelonephritis) in different countries, including those from the Infectious Diseases Society of America, rely on data derived from bacterial isolates from women, and tend to exclude men from their recommendations, most likely due to lack of evidence to guide recommendations. Furthermore, it's unclear whether the rate of antibiotic resistance in UPEC isolates from men is comparable to those from women, and whether this differs by age group and/or uro-clinical syndrome. However, the available limited studies seem to suggest that the antibiotic resistance rate of UPEC in men is higher than in women,[6] which could have implications for empiric treatment guidelines in men.

Biofilm production by UPEC is an important virulence factor,[7] as its formation creates an antibiotic impermeable zone, thus contributing to the development of resistance, as confirmed by previous studies demonstrating strong association between biofilm production and antibiotic resistance in women UPEC isolates.[8] However, little is known about the relationship between antibiotic resistance and biofilm production in urinary *E. coli* isolates from men, according to clinical syndrome and age group.

Therefore, we studied urinary *E. coli* isolates from men aged 0-60 years, with cystitis or pyelonephritis, from the same geographical area and time period, in order to define:

(i) the distribution of UPEC by clinical syndrome and hospitalization status

(ii) the prevalence of antibiotic resistance in relation to uro-clinical syndrome and age group; and,

(iii) finally, the association between biofilm production and antibiotic resistance.

Materials and methods

Study design

This was a prospective study carried over 3 years, incorporating 11 regional hospitals and 23 outpatient medical centers, in the Central West region of New South Wales (NSW), Australia (population, 180 000). Each of the participating physicians in the study received a standardized urine collection protocol, and the clinical diagnostic criteria for cystitis or pyelonephritis. During medical history taking and physical examination, the physicians recorded the following; deidentified patient information: age, clinical UTI syndrome, previous UTI history, and any known underlying host conditions.

Bacterial isolates and study subjects

Midstream or clean catch urine specimens were collected from qualifying men presenting to the participating centers. Based on a standardized uro-clinical diagnostic criteria, a diagnosis of cystitis or pyelonephritis required specific clinical manifestations, in addition to a urine culture yielding $\geq 10^8$ cfu/L of *E. coli*. Cystitis was defined clinically by frequency of urination, dysuria, and/or suprapubic tenderness, without fever or loin pain. And pyelonephritis was categorized by urinary symptoms, fever of $\geq 38^{o}$C, and flank pain, with or without nausea and/or vomiting. *E. coli* was identified by conventional biochemical tests and/or molecular tests as previously described.[9-10] The bacterial isolates were stored in 5% glycerol in trypticase soy broth at -70^{o}C until further use.

A total of 310 *E. coli* isolates from urine specimens of men (0-60 years old) with cystitis (n = 229) or pyelonephritis (n = 81), were studied. Of these, 189 (61%) were from the outpatient setting, whilst 121 (39%) were inpatients (hospitalized). These isolates were collected over a 3-year period (July 2011-2014), with only one isolate per subject included in the study. To minimize the possible confounding effect of some host characteristics on bacterial characteristics, patients with known diabetes mellitus, diarrhoea, antibiotic therapy in the last month, or urinary tract abnormalities, were excluded.

Antibiotic susceptibility

The *E. coli* isolates were tested for susceptibility to 14 antibiotic drugs according to the Clinical Laboratory Standards Institute (CLSI)-specified disk diffusion method,[11] using Neo-Sensitabs discs (Rosco, Taasrup, Denmark). The antibiotics tested were (disk content): amikacin (30 µg), amoxicillin-clavulanate (60 µg), ampicillin (25 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (10 µg), gentamicin (10 µg), imipenem (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (TMP-SMZ) (5 µg). To detect production of extended-spectrum β-lactamases (ESBLs), the double-disk diffusion test was used.[12] Resistant isolates were those that were resistant to ≥ 1 agent. The resistance score was the number of antibiotic classes (of the 13 classes studied) for which an isolate exhibited resistance to ≥ 1 representative agent. *E. coli* strain ATCC 25955 used as the control strain.

Detection of biofilm formation

The ability to form biofilm *in vitro* was assessed by a quantitative assay using a microtiter-plate test (Nunc, Roskilde, Denmark). Strains were grown on Brain Heart Infusion (BHI) agar, and colonies were re-suspended in a BHI broth (Oxoid, Basingstoke, UK) to reach the 0.5 suspension of McFarland's standard, and volumes of 200 µL of these cell suspensions were transferred to the wells of the microplate. For the negative control, an uninoculated BHI medium was used. After incubation (24 h at 37◦C), the adherent cells were washed three times using a saline solution and stained with a 0.1% crystal violet solution (Mikrochem, Pezinok, Slovakia). The adhering dye was dissolved with 30% acetic acid, and the optical density was measured at 570 nm in the Synergy HT Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA). For classification of the biofilm formed, we used the average optical density (OD) value and cut-off value (ODc) (defined as three standard deviations (SD) above the mean OD of the negative control). The final OD value of a tested strain was expressed as the average OD value of the strain reduced by the ODc value. For the interpretation of the results, the strains were divided into the categories described by Stepanovic *et al*. 2007:[13] OD ≤ ODc = non-biofilm producer; ODc < OD ≤ 2 × ODc = weak biofilm; 2 × ODc < OD ≤ × ODc = moderate and 4 × ODc < OD = strong biofilm producer.

Ethics approval

The project was approved by relevant institutional review boards (Charles Sturt University and Sydney West Area Health Service research committees). As clinical information for patients with UTI was provided anonymously by clinicians, patient consent was waived by both committees mentioned above.

Statistical analysis

Comparisons of proportions were tested using Fisher's exact test. Virulence and resistance score comparisons were tested using the Mann-Whitney U-test.

Results

Isolation rates of uropathogenic *Escherichia coli* by uro-clinical syndrome and age

The majority of the UPEC isolates were from the outpatient setting (189; 61%), with the various age groups generally uniformly distributed, and ranging from 26-37% (Table 1). In the inpatient setting, the 0-10 years age group was not represented, and the remaining three age groups were evenly represented, from 22-27%. Distribution of the isolates by uro-clinical syndrome showed that the majority of the isolates were from cystitis cases (74%), compared to only 26% for pyelonephritis cases ($P < 0.001$). Among the cystitis isolates, the distribution by age group was generally uniform, ranging from 22-30%. However, among the pyelonephritis isolates, the majority (65%) were from the 21-40 years age group, followed by the 41-60 years age group at 27%, and finally lowest in the 11-20 years age group at 12%. Similar to the cystitis isolates, the 0-10 years age group was not represented at all in the pyelonephritis isolates. A significantly higher proportion of isolates in the 0-10 years age group (30% vs 0%) and the 21-40 years age group (37% vs.27%) were confined to the outpatient setting than the inpatient one ($P < 0.001$ for both comparisons.

Distribution of antibiotic resistance by source

The prevalence of antibiotic resistance amongst the isolates was on average higher for pyelonephritis isolates than cystitis, with all the different antibiotics having higher resistance rates in the pyelonephritis than the cystitis cohort, most of which were statistically significant (Table 2). Amongst the cystitis isolates, the prevalence of antibiotic resistance ranged from 0% (ceftazidime) to 62% (tetracycline), whilst for pyelonephritis isolates, it ranged from 2% (amikacin) to 72% (tetracycline). Fluoroquinolone (norfloxacin or ciprofloxacin) resistance rate was significantly higher in pyelonephritis isolates at 19% compared to 7% for cystitis isolates ($P = 0.009$). ESBL production was higher in pyelonephritis isolates at 11% compared to 3% for cystitis isolates ($P = 0.012$). Likewise, multidrug resistance

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was higher amongst pyelonephritis isolates at 73% vs. 58% for cystitis isolates (P = 0.023). And finally, biofilm production was much more pronounced amongst pyelonephritis than cystitis isolates at 65% vs. 42% (P < 0.001).

Distribution of antibiotic resistance by age group amongst UPEC isolates from men

To determine antibiotic resistance by age group, we combined the cystitis and pyelonephritis isolates in order to have sufficient numbers in each group to increase statistical power (Table 3). The lowest resistance rate was observed in the 0-10 years age group, with the prevalence rate of most antibiotics tested being lowest in this cohort. Consequently, multidrug resistance and the resistance scores were lowest in this age group at 43% and 4 (0-7), compared to at least 55% and 6 (0-10) for the other groups, respectively (P = 0.015). This was followed by the 11-20 years age group, with the overall rate of multidrug resistance (57%) being significantly higher than that of the 0-10 years age group (39%) (P = 0.048). The highest rate of antibiotic resistance was confined to the 21-40 years age range, having the highest rate for each of the antibiotic tested, for all the cohorts. Consequently, multidrug resistance was highest in this cohort, with 69% of the isolates being resistant to more than one antimicrobial agent, compared to 39% for the 0-10 years age group, and 55% for the 41-60 years age group. Likewise, ESBL production was highest (10%) in the 21-40 years age range, followed by the 41-60 years cohort (5%), and lowest in the 0-10 years age group, with no ESBL detected. Consequently, antibiotic resistance scores were highest in the 21-40 age range, medium in the 41-60 age group, and lowest in the 0-10 years age group.

Multidrug resistance was much more common amongst pyelonephritis isolates with higher rates in each category compared to the cystitis isolates (Table 4). The most common multidrug resistance for both uro-clinical syndromes was the one involving 2-4 antibiotics, and was observed in 59% and 44% of pyelonephritis and cystitis isolates, respectively. A significant proportion of pyelonephritis isolates (16%) was resistant to at least 10 antibiotics, compared to 3% for cystitis isolates (P < 0.001). The majority of ESBL producers from pyelonephritis isolates (82%) were resistant to at least 10 antibiotics, in contrast to the majority in cystitis isolates (55%) which were resistant to between 5-9 antibiotics, *albeit* limited number of isolates in both groups.

Table 1 Prevalence of UPEC UTI by age group among 310 *Escherichia coli* isolates from men with cystitis or pyelonephritis

Age group (years)	[a]Number (%) of isolates				[b]P value	
	Cystitis (n = 229)	Pyelonephritis (n = 81)	Outpatient (n = 189)	Inpatient (n = 121)	[c]Cyst vs. [d]Pyelo	Outpatient vs. inpatient
0-10	56 (25)	0 (0)	56 (30)	0 (0)	< 0.001	< 0.001
11-20	69 (30)	10 (12)	50 (26)	29 (23)	0.002	0.689
21-40	50 (22)	53 (65)	70 (37)	33 (27)	< 0.001	< 0.001
41-60	54 (24)	22 (27)	50 (26)	26 (22)	0.105	0.346

[a]Data are numbers (%) of isolates (%) in each column.

[b]P values are shown, calculated by Fisher's exact test.

[c]Cyst, cystitis.

[d]Pyelo, pyelonephritis.

Table 2 Prevalence of antibiotic resistance among 310 *Escherichia coli* isolates from men with cystitis or pyelonephritis

Antibiotic	Source of isolates, no. (%)[a]		P value[b]
	Cystitis (n = 229)	Pyelo[c] (n = 81)	Cystitis vs. pyelo
Ampicilin	87 (38)	48 (59)	0.001
Amoxicillin-clavulanate	57 (25)	32 (39)	0.015
Cephalothin	83 (36)	46 (57)	0.002
TMP-SMZ[d]	32 (14)	20 (25)	0.037
Nitrofurantoin	7 (3)	5 (6)	0.311
Gentamicin	10 (4)	7 (8)	0.16
Amikacin	3 (1)	2 (2)	0.609
Norfloxacin	17 (7)	13 (16)	0.009
Ciprofloxacin	17 (7)	15 (19)	0.134
Imipenem	0 (0)	1 (2)	0.262
Nalidixic acid	50 (22)	23 (29)	0.286
Tetracycline	140 (61)	60 (74)	0.043
Ceftriaxone	7 (3)	4 (5)	0.486
Ceftazidime	0 (0)	2 (3)	0.068
ESBL producer[e]	11 (5)	11 (14)	0.012
Resistant to > 1 agent	133 (58)	59 (73)	0.023
Biofilm production	96 (42)	53 (65)	< 0.001
Resistance score; median (range)	5 (0-11)	9 (0-13)	0.021

[a]Data are numbers (%) of isolates resistant to individual antibiotic agents (%) in each column, except for last row.

[b]P values are shown where p < .05, calculated by Fisher's exact test, except for last row, where Mann Whitney-U test was used.

[c]Pyelo, pyelonephritis.

[d]TMP-SMZ, trimethoprim-sulfamethoxazole.

[e]ESBL, extended-spectrum beta-lactamase.

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Table 3 Distribution of antibiotic resistance in relation to age group among 310 *Escherichia coli* isolates from men with urinary tract infection

Resistance or other trait	Prevalence of trait, no. (column %)[a]				P value (Fisher's exact test)[b]		
	0-10 (n = 56)*	11-20 (n = 75)**	21-40 (n = 103)***	41-60 (n = 76)****	* vs. ***	** vs. ***	*** vs. ****
Ampicillin	11 (20)	23 (30)	55 (53)	29 (38)	< 0.001	0.004	0.05
Amoxicillin-clavulanate	10 (17)	25 (33)	44 (43)	20 (26)	0.002	0.217	0.028
Cephalothin	18 (32)	25 (33)	54 (52)	32 (42)	0.019	0.014	0.178
TMP-SMZ[c]	7 (13)	11 (15)	28 (27)	16 (21)	0.044	0.066	0.384
Nitrofurantoin	0 (0)	2 (3)	7 (7)	3 (4)	0.053	0.306	0.521
Gentamicin	1 (2)	3 (4)	9 (9)	4 (5)	0.1	0.364	0.562
Amikacin	0 (0)	0 (0)	4 (4)	1 (1)	0.3	0.138	0.4
Norfloxacin	4 (6)	2 (3)	29 (28)	5 (7)	0.002	< 0.001	< 0.001
Ciprofloxacin	4 (7)	5 (7)	27 (26)	10 (13)	0.003	< 0.001	0.04
Imipenem	0 (0)	0 (0)	1 (1)	0 (0)	1	1	1
Nalidixic acid	7 (13)	17 (22)	31 (30)	15 (20)	0.019	0.307	0.124
Tetracycline	22 (39)	29 (40)	93 (91)	56 (74)	< 0.001	< 0.001	0.004
Ceftriaxone	(1)	0 (0)	7 (7)	4 (5)	0.262	0.022	0.761
Ceftazidime	0 (0)	0 (0)	1 (1)	1 (1)	1	1	1
ESBL production[d]	0 (0)	2 (3)	10 (10)	2 (3)	0.015	0.075	0.074
Resistant to > 1 agent	22 (39)	43 (57)	72 (69)	42 (55)	0.001	0.012	0.041
Resistance score; median (range)	4 (0-7)	6 (0-10)	9 (0-14)	7 (0-12)	0.023	0.034	0.038

[a]Data are numbers (%) of isolates resistant to individual antibiotic agents (%) in each column, except for last row.

[b]P values are shown where p < .05, calculated by Fisher's exact test, except for last row, where Mann Whitney-U test was used.

[c]TMP-SMZ, trimethoprim-sulfamethoxazole.

[d]ESBL, extended-spectrum beta-lactamase

Table 4 Prevalence of multidrug resistance by uro-clinical syndrome

Number of antibiotics resistant to	Source of isolates, no. (%)[a]				[b]P value	
	Cystitis (n = 229)	Pyelo[c] (n = 81)	[d]ESBL producer cystitis (n = 11)	ESBL producer pyelonephritis (n = 11)	Cyst vs pyelo	ESBL Cyst vs pyelo
4-Feb	101 (4)	48 (59)	0 (0)	0 (0)	0.02	[e]NS
5-Mar	66 (29)	34 (42)	0 (0)	0 (0)	0.038	NS
7-Apr	92 (40)	45 (56)	1 (9)	0 (0)	0.019	NS
9-Jun	41 (18)	24 (30)	6 (55)	2 (9)	0.038	0.183
>10	7 (3)	13 (16)	4 (36)	9 (82)	< 0.001	0.081

[a]Data are numbers (%) of isolates resistant to individual antibiotic agents (%) in each column, except for last row.

[b]P values are shown where p < .05, calculated by Fisher's exact test, except for last row, where Mann Whitney-U test was used.

[c]Pyelo, pyelonephritis.

[d]ESBL, extended-spectrum beta-lactamase.

[e]NS, not significant

Discussion

Most studies on UTI epidemiology, and specifically UPEC pathogenesis, have largely concentrated on the characteristics of bacterial isolates from reproductive age women, as UTI occurs much more frequently in this host group. Consequently, there is a scarcity of literature on UTI epidemiology in men, which limits our understanding of UTI pathogenesis in this gender, including treatment options. The present study is one of a few that have analysed a high number of UPEC isolates from men from one region, collected consecutively over a period of 3 years, to gain insights into the antibiotic resistance pattern of UPEC isolates from men, by uro-clinical syndrome and age group.

Our findings show that, just like in reproductive age women, cystitis is the most common uro-clinical syndrome in men, with 74% of the isolates studied restricted to this condition. This is not a surprising finding as the majority of upper urogenital infections, including pyelonephritis, start as a lower urogenital infection, and depending on the bacterial characteristics of the invading strain, including antibiotic resistance, and host status, a select few can then ascend to cause pyelonephritis. Furthermore, just like in women, the majority of the UTI cases (61%) in the present study were confined to the outpatient setting, which is not surprising as most uncomplicated UTIs are generally mild and hence mostly handled by general practitioners in the outpatient setting. Likewise, a greater proportion of the pyelonephritis isolates were derived from the inpatient setting, which is to be expected as pyelonephritis is a more serious clinical condition requiring hospitalization in the majority of cases.[14]

Notably, the present study provides the first evidence within regional NSW Australia and elsewhere, of differences in antibiotic resistance rates by uro-clinical syndrome and age group, among urinary *E. coli* isolates in men without medical, or urological conditions predisposing to UTI. Specifically, the prevalence rate of resistance to most of the antibiotics tested, was on average, higher in pyelonephritic than cystitis isolates. Consequently, the antibiotic resistance score (median 9 (0-13 range vs. median 5 (0-11 range),

multidrug resistance (73% vs. 58%) and ESBL production (11% vs. 3%), were higher in pyelonephritis than cystitis isolates. Although a similar distribution has been described in UPEC isolates from females elsewhere,[15] including isolates from the same geographical location and time period to the present isolates,[16] the rate of antibiotic resistance in the present study was higher than in women for both uro-clinical syndromes. This can possibly be explained by the fact that on average, males tend to present more often with more complicated type of UTI which may be associated with more antimicrobial-resistant bacterial strains.[17]

The significant difference in the distribution of antibiotic resistance by age group, with the 21-40 years age group exhibiting the highest rate, followed by the 41-60 years age group, and least in the 0-10 years age group, has, to the best of our knowledge, not been described before in UPEC isolates from men, and even in reproductive age women. This finding suggests a possible heterogenous clustering of individual and combined resistance phenotypes by age group, perhaps via shared antimicrobial drug resistance elements, including plasmids and integrons, and/or host group characteristics, as previously observed.[18] These findings have important implications in effective UTI management practices for the central NSW region, and by extension similar jurisdictions in Australia, and possibly elsewhere. Firstly, due to the stringent selection criteria implemented in the study, the findings are more likely to be representative of the typical male patients in the 0-60 years age group in the Central West region of NSW, Australia, who present with uncomplicated cystitis and pyelonephritis. As such, findings from this study may be much more useful in formulating recommendations for guiding empirical treatment in male UTI in the region, rather than relying on susceptibility data obtained from pathology labs in the region as that data can be confounded by many factors. Secondly, these findings reinforce the importance of having an antibiotic resistance surveillance system as an adjunct to clinical guidelines for use in management of UTIs, to keep track of new developments in antibiotic susceptibility patterns.

The prevalence of antibiotic resistance among the present isolates, with 42% and 73% of the isolates being resistant to one or more antibiotics, for cystitis and pyelonephritis, respectively, is modest compared to other jurisdictions with much higher rates, *albeit* limited number of studies and isolates.[19] Over the last 15 to 20 years, antibiotic surveillance programs have shown reductions in the use of antibiotic drugs in Australia,[20] Slovakia,[21] and Sweden.[22] Thus, our results probably reflect more conservative antibiotic prescribing and consumption practices in Australia than in many other countries. Unfortunately, similar previous studies describing associations between age group and antimicrobial resistance rates in males, with which to compare the current data, are quite scarce in literature. Thus, the present findings provide some baseline data for future comparative studies in regional NSW, Australia, and for other countries, and also contribute relevant data for inclusion in literature for worldwide use. Evidently, the described high rate of multidrug resistance among the urinary *E. coli* isolates in the NSW region of Australia, suggests that monitoring these phenotypes is important and should be a consideration as the guidelines for the empiric treatment of UTIs evolve.

Guidelines for treatment of uncomplicated cystitis, including those issued by the Infectious Diseases Society of America (IDSA), recommend use of TMP-SMZ as a first line therapy in geographical locales with resistance rates of about 10-15%. The increased *in vitro* resistance rate to TMP-SMZ of between 13-27% among the different age groups in the current study, which is above the local threshold, is concerning as it is associated with treatment failure in both cystitis and pyelonephritis. To deal with this therapeutic challenge requires use of alternative treatment, such as a fluoroquinolone, which, according to findings in the present study, is also a challenge as 7-26% of the isolates in the different age groups were resistant to this agent.

The ability to produce extended-spectrum beta-lactamases (ESBLs) and biofilm is an important defence mechanism for bacteria to survive the harsh environment of antibiotic presence. As such, bacterial strains exhibiting these characteristics, which confer them with capacity to resist a wide variety of antibiotics, are naturally selected to thrive in the presence of antimicrobial drugs, and hence may proliferate further and spread in the community. A significant proportion of our pyelonephritis (14%), and 5% of cystitis isolates, were ESBL producers, which, although is low compared to rates in other regions of the world,[23] is still a public health concern as these strains can spread rapidly in the community as has been demonstrated with ESBL positive ST131 *E. coli* strains.[24-25] The proportion of ESBL producers in the present study is comparable to rates in UPEC isolates from reproductive age women in the same geographical location and collected during the same period, at 9% and 5%, for pyelonephritis and cystitis isolates, respectively[16]. However, in contrast to these findings, high rates of ESBL prevalences of greater than 50% have been described in several developing countries.[26-28] In addition, relatively high rates of ESBLs have also been reported in some developed countries, including rates of 53% and 44% in inpatients, and outpatients, respectively.[29] These differences in rates of ESBLs most probably reflect the effectiveness of policies and procedures around antibiotic stewardship in different jurisdictions worldwide.

Biofilm production by bacteria, which has been extensively demonstrated in urine catheter infections,[30] is one key strategy employed by UPEC to limit the effectiveness of antibiotics. Biofilms help not only in the transfer of plasmid encoding resistance genes such as ESBL to other organisms via conjugation, but also resist immune clearance.[31] A highly significant proportion of our UTI isolates were *in vitro* biofilm producers, specifically 65% and 42% of the pyelonephritis and cystitis isolates, respectively, implying that in the right host setting, such strains can induce biofilm formation and contribute to development of antibiotic resistance. This is quite concerning as previous studies indicate that multidrug resistance is strongly associated with biofilm production. [32-33] Bacterial biofilms play an important role in UTI pathogenesis, being implicated in persistent infections leading to recurrences and relapse. A better understanding of the factors contributing to biofilm formation in UPEC may be important in the conception of new therapeutic options for UTI treatment. We did not classify our uro-clinical isolates into prostatitis or not, but suspect that a reasonable proportion were from cases of prostatitis. It has been previously observed that UPEC causing prostatitis presented a higher capacity to form "*in vitro*" biofilm than those causing cystitis and pyelonephritis.[34] The increased capacity to form biofilm of these strains could be a possible explanation for the persistence of such strains in the prostatic secretory system. Furthermore, a study of women with recurrent UTI demonstrated that uropathogens can persists within the bladder tissue in underlying epithelial cells or creating pod-like bulges on the bladder surface being a source of recurrent UTI.[35]

The majority of our isolates that were biofilm producers originated from the inpatient setting (data not shown), a finding that is to be expected as the hospital environment is known to select for the proliferation of multidrug resistant strains. Studies have estimated that about 65% of microbial infections are associated with biofilms,[36] and also that biofilm cells are 100 to 1 000 times more resistant to antimicrobial agents than planktonic cells. Previous studies, mostly

from females, have also shown that biofilm production is significantly higher in *E. coli* ESBL producing strains,[33] including a recent study by Ramos et al.,[37] which showed that antibiotic resistance and ESBL production were associated with expression of biofilm components, curli and cellulose.

The overall antibiotic resistance rate amongst the male isolates, with at least 58% of the isolates resistant to one or more drugs, was comparable to that of isolates from females collected from the same geographical location and time period, at 52%.[38] However, the prevalence of antibiotic resistance for most antibiotics tested were lower than has been reported from North America and Europe.[39-40] In addition, contrary to our findings, higher prevalences of quinolone and FQ resistance have been reported among *E. coli* cystitis isolates than pyelonephritis isolates.

The strengths of this study include the large sample size of 310 UPEC isolates well characterized by uro-clinical syndrome (cystitis, pyelonephritis), and collected consecutively from the same geographical location and time period. Study limitations include the use of multiple comparisons which increased the likelihood of type-I errors, as well as pooling some isolates together into groups to increase statistical power. In addition, we classified isolates into outpatient *vs.* inpatient based on the facility at which the specimen was collected. However, in some remote places, the hospitals are more akin to a primary healthcare facility in a metropolitan setting than a tertiary hospital.

Conclusion

In conclusion, we documented a significant difference in the distribution of antibiotic resistance by age group amongst UPEC isolates from men in regional NSW, Australia, in the 0-60 years age group; being highest in the 21-40 years group and lowest in the 0-10 years age group. Furthermore, biofilm production was much more pronounced in the pyelonephritis than cystitis isolates. Findings from this study can be of utility in evidence best practice recommendations for empirical therapy of UTI in the region, and can also be used as a base rate for future studies in the region and elsewhere. Given that the majority of therapy for UTIs is empirical, and that urinary tract pathogens are demonstrating increasing antimicrobial resistance, continuously updated data on antimicrobial susceptibility patterns would be beneficial to guide empiric treatment.

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Conflicts of interest

None to declare.

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