PL Ho 何柏良
KY Yuen 袁國勇
RMK Lam 林文健
KM Kam 甘啟文

Antimicrobial resistance among uropathogens causing cystitis in women

Key Messages

1. Among *Escherichia coli* from adult women with acute cystitis, the rates of antimicrobial resistance were 52.8% for ampicillin, 29.5% for co-trimoxazole, and 12.9% for ciprofloxacin. Nitrofurantoin and fosfomycin remain active against >90% of the isolates.
2. The respective age-stratified rates for co-trimoxazole and ciprofloxacin resistance were 26.4% and 9.6% for women aged 18-50 years, and 35.5% and 19.4% for women aged ≥51 years. Being aged ≥51 years (Odds ratio [OR]=2.3, 95% confidence interval [CI]=1.1-4.8, P=0.02) and receipt of recent antibiotic treatment (OR=2.5, 95% CI=1.1-5.8, P=0.03) were significantly associated with fluoroquinolone resistance.
3. Ampicillin and co-trimoxazole should not be used as first-line agents for empirical treatment of acute cystitis. As fluoroquinolone resistance was less than 10% among isolates obtained from younger women, these agents are still useful for empirical treatment. Nonetheless, the high rates of fluoroquinolone resistance among women who were older or who had received antibiotic treatment recently indicates the need to consider alternatives such as nitrofurantoin, fosfomycin and amoxicillin-clavulanate as initial therapy.
4. Molecular analysis of the multidrug resistant strains showed that they were genetically diverse, with no evidence of epidemic strains. Nonetheless, resistant strains possess virulence traits distinct from susceptible isolates, suggesting that they may evolve from other sources and not by acquisition of resistance mutations in susceptible isolates from humans.

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Department of Microbiology, The University of Hong Kong
PL Ho, KY Yuen
Department of Health, Hong Kong SAR, China: Centre for Health Protection
RMK Lam
Public Health Laboratory Centre
KM Kam

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Principal applicant and corresponding author:
Prof PL Ho
Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China
Tel: (852) 2855 4892
Fax: (852) 2855 1241
Email: plho@hkucc.hku.hk

Introduction

Cystitis or urinary tract infection (UTI) affects one-third of women at some stage during their lifetime. In one quarter to one third of such patients, the infection is recurrent. The microbial aetiology of UTI is well established. *Escherichia coli* is the predominant pathogen (80%); the antibiotic treatment is mainly determined by the prevailing antimicrobial resistance of this organism. First-line agents recommended include ampicillin, co-trimoxazole and fluoroquinolones, but resistance rates to these first-line agents among community isolates of *E coli* in Hong Kong were reported to be 66%, 57%, and 23%, respectively.[1] Whether alternative agents are needed for some or all women is unknown, because all previous local studies failed to take into account patient demographics, types of infection and the clinical settings. The present study was therefore conducted to address this issue.

This study evaluated antimicrobial resistance rates of *E coli* and other pathogens isolated from patients with community-acquired cystitis. The epidemiological relation of the antibiotic-resistant isolates was evaluated using molecular typing. The correlation between bacterial virulence factors and antimicrobial resistance was also assessed.

Methods

This prospective cross-sectional study was conducted from January 2006 to June 2008. It comprised 54 centres including general practitioner offices, general outpatient clinics and emergency departments. Adult women (aged ≥18 years) diagnosed with uncomplicated cystitis were enrolled. Patients with loin pain, fever, renal stones and indwelling urinary catheters were excluded. A standardised questionnaire was used to collect patient demographics, history, and underlying medical details. A mid-stream urine sample was obtained for culture and processed by standard methods. Bacterial identification and antimicrobial susceptibility testing was performed using established methods.[2]

Beta-lactamases related to the CTX-M families were sought by polymerase chain reaction and sequencing, using primers previously described.[2] Selected isolates were studied by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA (Amersham Pharmacia Biotech, Little Chalfont, UK), and patterns were analysed with Gelcompar II software (Applied Maths). Isolates were tested for 30 virulence-associated traits using established multiplex polymerase chain reactions.[3]

The Chi-squared test, Fisher's exact test, or Student's *t*-test were used for statistical analysis. A two-tailed P value of <0.05 was considered significant.

Results

Patient demographics and antimicrobial susceptibilities

A total of 592 patients with uncomplicated cystitis were recruited. Among these, 359 were enrolled from general practitioner offices, 101 from general outpatient clinics, and 132 from emergency departments (Table 1). In 237 (40%) of the patients, the urine cultures did not grow any bacteria, and in three (0.5%) others

the cultures were contaminated. Thus, the study population consisted of 352 (59.5%) patients, whose urine samples grew a single uropathogen on culture. The mean age of these patients was 44.9 years (standard deviation, 16.3 years); most (96.8%) of whom were Chinese. Underlying comorbidities were present in 38 patients, the commonest being diabetes mellitus (n=20), hypertension (n=6) and heart disease (n=4).

The rates of antibiotic-resistant *E coli* were 52.8% for ampicillin, 45.4% for nalidixic acid, 29.5% for co-trimoxazole, and 12.9 for ciprofloxacin (Table 2). Almost all isolates (>90%) were susceptible to nitrofurantoin and fosfomycin. Fourteen (5.2%) of the isolates were extended-spectrum beta-lactamases (ESBL) producers. The point prevalence of ESBL isolates in these samples was 2.8% (5/178) for women aged 18-50 years, and 9.7% (9/93) for women aged ≥51 years. The ESBL producer rate was 20.8% among triple drug resistant strains (defined as co-resistance to ampicillin, ciprofloxacin, and co-trimoxazole) versus 3.6% among non–triple drug resistant strains (P<0.001). The resistance rates to ampicillin (60.2% vs 48.9%), nalidixic acid (54.8% vs 40.4%), ciprofloxacin (19.4% vs 9.6%), and co-trimoxazole (35.5% vs 26.4%) among women aged ≥51 years were higher than those in younger women (aged 18-50 years), although only the difference for nalidixic acid

Table 1 Epidemiological characteristics of 352 patients with cystitis

Epidemiological characteristics	No. (%) of patients with cystitis		
	All (n=352)	18-50 years old (n=235)	≥51 years old (n=117)
Underlying comorbidities	38 (10.8)	11 (4.7)	27 (23.1)
Prior cystitis	94 (26.7)	53 (22.6)	41 (35)
Recent antibiotic use	46 (13.1)	28 (11.9)	18 (15.4)
Presentation setting			
General practitioner office	61 (17.3)	177 (70.2)	75 (29.8)
General outpatient clinic	39 (11.1)	18 (46.2)	21 (53.8)
Emergency department	252 (71.6)	40 (65.6)	21 (34.4)
Uropathogen			
Escherichia coli	271 (77)	178 (75.7)	93 (79.5)
Other *Enterobacteriaceae*	50 (14.2)	35 (14.9)	15 (12.8)
Staphylococci	18 (5.1)	15 (6.4)	3 (2.6)
Others	13 (3.7)	7 (3)	6 (5.1)

Antibiotic	% of patients					
	E coli (n=271)			Other *Enterobacteriaceae* (n=50)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin	40.6	6.6	52.8	26.5	0	73.5
Amoxicillin-clavulanate	84.9	13.3	1.8	79.6	8.2	12.2
Nalidixic acid	52.8	1.8	45.4	75.5	16.3	8.2
Ciprofloxacin	87.1	0	12.9	95.9	2	2
Co-trimoxazole	69.4	1.1	29.5	83.7	0	16.3
Nitrofurantoin	92.3	1.1	6.6	30.6	14.3	55.1
Fosfomycin	98.2	0	1.8	91.8	2	6.1

Table 3 Distribution of bacterial characteristics among two *Escherichia coli* subsets

Bacterial characteristics*	No. (%) of isolates*		P value
	Susceptible (n=42)	Multidrug resistant (n=34)	
Adhesin VF genes			
focG (F1C fimbriae)	**7 (21)**	1 (3)	0.03
papA (P-fimbriae structural subunit)	**26 (77)**	18 (53)	0.02
papC (P-fimbriae assembly subunit)	**27 (79)**	19 (56)	0.02
papEF (P-fimbriae tip pilin)	**24 (71)**	13 (38)	0.01
papG (P-fimbriae adhesin molecule)	**27 (79)**	17 (50)	0.01
papG allele III (P-adhesin variant III)	**22 (65)**	9 (27)	<0.01
sfa/foc DE (S and F1C fimbriae)	**24 (71)**	13 (38)	0.01
sfaS (S fimbriae)	**15 (44)**	8 (24)	0.05
Toxin VF genes			
cnf1 (cytotoxic necrotising factor)	**26 (77)**	12 (35)	<0.01
hlyA (haemolysin)	**26 (77)**	7 (21)	<0.01
Capsule polysaccharide VF genes			
K1 (K1 kpsMT II variant)	4 (12)	**11 (32)**	0.05
Siderophore and miscellaneous VF genesa			
cvaC (Colicin V)	1 (3)	**5 (15)**	0.04
iutA (aerobactin receptor)	3 (9)	**18 (53)**	<0.01
traT (serum resistance)	17 (50)	**27 (79)**	0.02
Phylogenetic group			
E coli reference group A	3 (9)	3 (9)	NS
E coli reference group B	0 (0)	2 (6)	NS
E coli reference group B2	29 (85)	26 (76)	NS
E coli reference group D	2 (6)	3 (9)	NS

* Virulence factors are shown only if P≤0.05. Boldface indicates those factors occurred in higher frequencies. Multidrug resistant is defined as co-resistance to three or more drugs: ampicillin, ceftriaxone, ciprofloxacin, co-trimoxazole, fosfomycin, gentamicin, nitrofurantoin and tetracycline. Susceptible subset (control) isolates were sensitive to all eight drugs.

and ciprofloxacin reached statistical significance.

Risk factor for antibiotic-resistant Escherichia coli

Antibiotic use in the past 6 weeks significantly increased the risk of infection by co-trimoxazole-resistant *E coli* (odds ratio [OR]=2.8, P=0.003) and ciprofloxacin-resistant *E coli* (OR=2.3, P=0.03). Resistance rates to co-trimoxazole and ciprofloxacin among patients with a history of recent antibiotic use were 50% and 23.7%, respectively. Older age (≥51 years) was also associated with infection by ciprofloxacin-resistant *E coli*.

Molecular analysis

A subset of 68 isolates including 34 multidrug resistant (MDR) isolates and 34 susceptible controls were analysed further by PFGE and virulence factor analysis. Based on PFGE, the MDR isolates had highly diverse banding patterns, and there was little overlap with the susceptible controls. All 14 ESBL-positive isolates were also analysed by PFGE and noted to be genetically distinct and not clonally related. Virulence profiling showed that adhesin and toxin genes were significantly more frequent among the susceptible subset than the MDR subset. In contrast, the K1 (capsule), *iutA* (siderophore), *cvaC* (colicin V) and *traT* (serum resistances) genes were more commonly found in the MDR strains (Table 3). Resistance to ciprofloxacin but not ampicillin and co-trimoxazole was associated with lower aggregate VF scores.

In the conjugation experiments, the ESBL phenotype could be transferred from five of the 14 isolates. The frequency of transfer was 10^{-3} to 10^{-2} per donor cells. Polymerase chain reaction and sequencing showed that the presence of CTX-M-14 in 11 isolates, CTX-M-24 in two isolates, and CTX-M-9 in one isolate.

Discussion

Since the early and mid-1990s in Hong Kong, resistance rates to ampicillin and co-trimoxazole reached 50-60% and 10-20%, respectively. In 1991, the rate of fluroquinolone resistance among community *E coli* was 6%. Our data showed that ampicillin resistance remains at the same level but fluoroquinolone and co-trimoxazole resistance has increased substantially. Nonetheless, the fluoroquinolone (12.9%) and co-trimoxazole (29.5%) resistance rates were substantially lower than those reported in the Department of Health sentinel surveillance (21-26% and 37-46%, respectively) during 2005-2007. The spuriously higher rates of resistance in the Department of Health figures were likely a result of bias from excessive inclusion of elderly patients and those with complicated infections.

Our data documents the spread of ESBL producers among patients with UTIs. The detection of ESBL isolates from young women is a concern because such community-associated ESBL isolates were previously reported mainly from older women. In agreement with our recent work,[2] CTX-M-14 is the predominant enzyme type among the ESBL-producers. As the isolates were not clonally related, the CTX-M determinant could possibly have spread through dissemination of epidemic plasmids. Additional investigation into the plasmid epidemiology is warranted. As food animals have been suggested to be important reservoirs for the CTX-M enzymes, future work should focus on the relation between the CTX-M encoding plasmids in human and animal isolates.[4]

In accordance with current guidelines,[5] the empirical use of co-trimoxazole as first-line therapy for women with community-acquired UTIs should be avoided. For younger women with cystitis, fluoroquinolones remain valuable as first-line agents. As ciprofloxacin-resistant isolates usually prevail in post-menopausal women, drugs other than fluoroquinolones may need to be re-considered for this patient population, especially those with a history of recent antibiotic treatment. Nitrofurantoin and fosfomycin remain active against most *E coli* isolates. In using nitrofurantoin, the duration of treatment should be extended to 5 to 7 days as there is insufficient data to support the efficacy of 3-day therapy. In selection of alternative agents, *in vitro* activity, clinical efficacy and side effects are important considerations. In future revision of management guidelines, we suggest inclusion of age as a variable in the approach to empirical therapy.

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