ASM Microbe 2018
SATURDAY - 416

www.can-r.ca

In Vitro Susceptibility of *Escherichia coli* Isolated from Urine Specimens of Canadian Outpatients from 2007-2016 to First- and Second-Line Empiric Oral Antimicrobial Agents

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Abstract

Background: *Escherichia coli* are responsible for 75-90% of uncomplicated urinary tract infections. The Infectious Diseases Society of America (IDSA) currently recommends an empiric regimen of five-days of nitrofurantoin, a three-day course of double-strength trimethoprim-sulfamethoxazole (SXT) in settings where the prevalence of SXT resistance is <10-20%, or a 3g single dose of fosfomycin trometamol (FOS) for treating acute bacterial cystitis in otherwise healthy adult non-pregnant females; fluoroquinolones and oral β-lactams are second-line therapies.
Methods: Isolates of *E. coli* were cultured from urine specimens of outpatients presenting to medical clinics and hospital emergency departments (EDs) for care and submitted to the annual CANWARD surveillance study from 2007 to 2016. FOS AST was performed using CLSI agar dilution testing (MHA supplemented with 25 µg/ml of glucose-6-phosphate; M100 27th edition [2017]); all other antibacterial agents were tested using the CLSI broth microdilution method. MICs were interpreted using M100 27th edition (2017) criteria. **Results:** See Table 2. **Conclusion:** Current (2016) *in vitro* susceptibilities of urinary isolates of *E. coli* to SXT (77.7%) and CIP (79.9%), two frequently prescribed empiric agents for outpatient urinary tract infections, appear compromised in comparison to NIT (97.8% susceptible) and FOS (99.3% susceptible). ESBL rates among isolates of *E. coli* increased from 0.8% in 2007 to 10.1% in 2016 while AmpC annual frequencies decreased from 3.2% in 2008 to 0.7% in 2016.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections of humans. Urine culture is the gold standard for the diagnosis of UTI (1). *Escherichia coli* are responsible for 75-90% of uncomplicated UTIs (2). An uncomplicated UTI is defined as a symptomatic bladder infection occurring in a female with a functionally normal urinary tract. The Infectious Diseases Society of America (IDSA) currently recommends an empiric regimen of five-days of nitrofurantoin, a three-day course of double-strength trimethoprim-sulfamethoxazole in settings where the prevalence of trimethoprim-sulfamethoxazole resistance is <10-20%, or a 3g single dose of fosfomycin trometamol for treating acute bacterial cystitis in otherwise healthy adult non-pregnant females; fluoroquinolones and oral β-lactams are second-line therapies (3).

Materials and Methods

Bacterial Isolates

The isolates of *E. coli* tested were cultured from urine specimens of outpatients attending medical clinics and hospital emergency departments and submitted to the annual CANWARD surveillance study from 2007 to 2016 (4). Primary isolate identification was performed by the submitting clinical microbiology laboratory site. If an isolate identification made by the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) using morphological characteristics and spot tests (5) was not consistent with that provided by the submitting site, the isolate was removed from the study.

Antimicrobial Susceptibility Testing

Fosfomycin antimicrobial susceptibility testing was performed using CLSI agar dilution testing (Mueller-Hinton agar supplemented with 25 µg/ml of glucose-6-phosphate); all other antibacterial agents were tested in cation-adjusted Mueller-Hinton broth using in-house-prepared 96-well broth microdilution panels according to CLSI guidelines (6). Quality control was performed following CLSI recommendations and minimum inhibitory concentrations (MICs) were interpreted using CLSI M100 breakpoints (6). ESBLs were identified and confirmed following CLSI guidelines (6) and multidrug-resistant (MDR) isolates were defined using a published guideline (7).

Results

Table 1. Prevalence of bacterial species isolated from urine specimens of outpatients collected by clinical microbiology laboratories in Canada from 2007 to 2016

	n (% of total n)							
Year (total n)	*E. coli*	*Klebsiella spp.*	*Proteus spp.*	*Enterobacter spp.*	Other Enterobacteriaceae	*P. aeruginosa*	*Enterococcus spp.*	*Staphylococcus spp.*
2016 (215)	139 (64.7)	27 (12.6)	7 (3.3)	6 (2.8)	3 (1.4)	4 (1.9)	15 (7.0)	9 (4.2)
2015 (228)	129 (56.6)	29 (12.7)	10 (4.4)	4 (1.8)	7 (3.1)	3 (1.3)	17 (7.5)	20 (8.8)
2014 (207)	130 (62.8)	19 (9.2)	12 (5.8)	2 (1.0)	4 (1.9)	5 (2.4)	24 (11.6)	6 (2.9)
2013 (255)	146 (57.3)	33 (12.9)	8 (3.1)	11 (4.3)	8 (3.1)	5 (2.0)	24 (9.4)	14 (5.5)
2012 (203)	119 (58.6)	26 (12.8)	9 (4.4)	6 (3.0)	8 (3.9)	5 (2.5)	17 (8.4)	10 (4.9)
2011 (244)	145 (59.4)	27 (11.1)	9 (3.7)	5 (2.0)	9 (3.7)	3 (1.2)	20 (8.2)	15 (6.1)
2010 (441)	271 (61.5)	42 (9.5)	12 (2.7)	12 (2.7)	11 (2.5)	6 (1.4)	55 (12.5)	20 (4.5)
2009 (471)	310 (65.8)	46 (9.8)	15 (3.2)	11 (2.3)	11 (2.3)	5 (1.1)	37 (7.9)	17 (3.6)
2008 (434)	285 (65.7)	29 (6.7)	18 (4.1)	7 (1.6)	13 (3.0)	8 (1.8)	46 (10.6)	20 (4.6)
2007 (601)	361 (60.1)	59 (9.8)	19 (3.2)	6 (1.0)	19 (3.2)	11 (1.8)	70 (11.6)	32 (5.3)
All (3,299)	2,035 (61.7)	337 (10.2)	119 (3.6)	70 (2.1)	93 (2.8)	55 (1.7)	325 (9.9)	163 (4.9)

Table 2. *In vitro* activities of first- and second-line empiric oral antimicrobial agents against outpatient urine isolates of *E. coli* collected by clinical microbiology laboratories in Canada from 2007 to 2016

	MIC_{90} (µg/ml) / % Susceptible					
Year (n)	SXT	Nitrofurantoin	Fosfomycin	Cephalexin[a]	AMC	Ciprofloxacin
2016 (139)	>8/77.7	16/97.8	4/99.3	>128/88.5	16/79.1	>16/79.9
2015 (129)	>8/73.6	16/97.7	2/99.2	16/92.2	16/83.7	>16/77.5
2014 (130)	>8/71.3	16/98.5	2/100	>128/87.6	16/83.7	>16/78.3
2013 (146)	>8/80.1	32/97.5	2/100	8/94.5	16/86.3	16/84.2
2012 (119)	>8/77.3	32/96.6	4/99.2	8/94.1	16/79.0	>16/83.2
2011 (145)	>8/70.8	32/97.2	2/99.3	64/88.3	16/86.8	>16/73.8
2010 (271)	>8/75.6	32/96.7	4/99.6	4/97.0	16/82.3	>16/83.0
2009 (310)	>8/74.2	32/97.9	4/98.6	8/95.8	8/94.8	>16/85.8
2008 (285)	>8/76.8	32/96.5	16/97.7	8/91.9	8/97.5	>16/83.5
2007 (361)	>8/84.5	32/97.7	2/100	8/96.4	8/97.3	>16/88.1
All (2,035)	>8/77.0	32/97.3	4/99.3	8/93.6	16/87.9	>16/83.0

Abbreviations: SXT, trimethoprim-sulfamethoxazole; AMC, amoxicillin-clavulanate. [a] Cephalexin susceptibility predicted by cefazolin MIC ≤16 µg/ml.

Table 3. ESBL and AmpC rates among outpatient urine isolates of *E. coli* collected by clinical microbiology laboratories in Canada from 2007 to 2016

Year (n)	% ESBL	% AmpC
2016 (139)	10.1	0.7
2015 (129)	5.4	0.8
2014 (130)	10.8	0.8
2013 (146)	3.4	2.1
2012 (119)	1.7	1.7
2011 (145)	9.0	2.1
2010 (271)	1.8	1.1
2009 (310)	1.6	1.6
2008 (285)	3.2	3.2
2007 (361)	0.8	NA
All (2,035)	3.8	1.4

Table 4. *In vitro* activities of first- and second-line empiric oral antimicrobial agents against outpatient urine isolates of *E. coli* collected by clinical microbiology laboratories in Canada from 2007 to 2016 stratified by resistance profiles

		(µg/ml)								(µg/ml)					
E. coli phenotype (n)	Antimicrobial agent	MIC_{50}	MIC_{90}	MIC range	% S	% I	% R	*E. coli* phenotype (n)	Antimicrobial agent	MIC_{50}	MIC_{90}	MIC range	% S	% I	% R
All (2,035)	SXT[a]	≤0.12	>8	≤0.12->8	77.0	NA	23.0	AMC Non-Susceptible (207)	SXT	2	>8	≤0.12->8	50.7	NA	49.3
	Nitrofurantoin	16	32	≤1-256	97.3	1.7	1.0		Nitrofurantoin	16	32	≤1-256	93.9	3.5	2.6
	Fosfomycin	≤1	4	≤1->512	99.3	0.6	0.1		Fosfomycin	≤1	4	≤1-64	100	0	0
	Cephalexin[b]	2	8	≤0.5->128	93.6	NA	6.4		Cephalexin	8	>128	1->128	70.5	NA	29.5
	AMC[c]	4	16	≤0.06->32	87.9	9.2	2.9		AMC	16	32	16->32	0	75.8	24.2
	Ciprofloxacin	≤0.06	>16	≤0.06->16	83.0	0.1	16.9		Ciprofloxacin	0.12	>16	≤0.06->16	59.9	0	40.1
SXT Non-Susceptible (468)	SXT	>8	>8	4-8	0	NA	100	Ciprofloxacin Non-Susceptible (346)	SXT	>8	>8	≤0.12->8	41.3	NA	58.7
	Nitrofurantoin	16	32	≤1-256	93.7	3.5	2.8		Nitrofurantoin	16	32	≤1-256	90.9	5.7	3.4
	Fosfomycin	≤1	4	≤1-128	98.9	1.1	0		Fosfomycin	≤1	8	≤1->512	96.8	2.8	0.4
	Cephalexin	4	>128	≤0.5->128	85.3	NA	14.7		Cephalexin	4	>128	1->128	76.6	NA	23.4
	AMC	8	16	1->32	75.7	20.5	3.8		AMC	8	16	1->32	73.2	21.6	5.2
	Ciprofloxacin	0.25	>16	≤0.06->16	56.6	0.5	42.9		Ciprofloxacin	>16	>16	2->16	0	0.6	99.4
Nitrofurantoin Non-Susceptible (46)	SXT	>8	>8	≤0.12->8	45.7	NA	54.3	ESBL-positive (77)	SXT	>8	>8	≤0.12->8	33.8	NA	66.2
	Nitrofurantoin	64	256	64-256	0	63.0	37.0		Nitrofurantoin	16	64	≤1-256	86.8	9.3	3.9
	Fosfomycin	2	16	≤1-128	97.6	2.4	0		Fosfomycin	2	4	≤1-128	96.1	3.9	0
	Cephalexin	4	>128	1->128	73.9	NA	26.1		Cephalexin	>128	>128	16->128	1.3	NA	98.7
	AMC	8	16	1-32	73.3	24.5	2.2		AMC	8	16	4->32	59.7	32.5	7.8
	Ciprofloxacin	16	>16	≤0.06	41.3	0	58.7		Ciprofloxacin	>16	>16	≤0.06->16	18.2	0	81.8
Fosfomycin Non-Susceptible (11)	SXT	≤0.12	>8	≤0.12->8	63.6	NA	36.4	AmpC-positive (28)	SXT	0.25	>8	≤0.12->8	64.3	NA	35.7
	Nitrofurantoin	16	32	4-64	90.9	9.1	0		Nitrofurantoin	16	32	4-256	96.4	0	3.6
	Fosfomycin	128	128	128->512	0	90.9	9.1		Fosfomycin	2	8	≤1->512	96.4	0	3.6
	Cephalexin	2	>128	1->128	63.6	NA	36.4		Cephalexin	64	>128	≤0.5->128	35.7	NA	64.3
	AMC	4	8	2-8	100	0	0		AMC	32	>32	1->32	25.0	21.4	53.6
	Ciprofloxacin	>16	>16	≤0.06->16	18.2	0	81.8		Ciprofloxacin	0.12	>16	≤0.06->16	57.1	0	42.9
Cephalexin Non-Susceptible (131)	SXT	>8	>8	≤0.12->8	47.3	NA	52.7	MDR (107)[d]	SXT	>8	>8	≤0.12->8	15.9	NA	84.1
	Nitrofurantoin	16	32	≤1-256	90.2	5.7	4.1		Nitrofurantoin	16	64	2-256	82.2	12.2	5.6
	Fosfomycin	2	4	≤1->512	96.6	2.6	0.8		Fosfomycin	2	8	≤1->512	93.5	5.6	0.9
	Cephalexin	>128	>128	32->128	0	NA	100		Cephalexin	128	>128	2->128	33.6	NA	66.4
	AMC	8	32	≤0.06->32	50.4	26.8	22.8		AMC	16	32	1->32	34.6	47.6	17.8
	Ciprofloxacin	>16	>16	≤0.06->16	38.2	0	61.8		Ciprofloxacin	>16	>16	≤0.06->16	4.7	0	95.3

[a] SXT, trimethoprim-sulfamethoxazole; [b] Cephalexin susceptibility predicted by cefazolin MIC ≤16 µg/ml; [c] AMC, amoxicillin-clavulanate; [d] MDR, non-susceptible to ≥3 agents from different antimicrobial classes (7).

Discussion

- *E. coli* was the most common urinary pathogen isolated from 2007 to 2016, accounting for between 56.6% and 65.8% of isolates per annum (mean 61.7%) (P>0.5) (Table 1).
- *Enterobacteriaceae* accounted for between 77.2% and 84.7% of isolates per annum (mean 80.4%) (P>0.5) (Table 1).
- Gram-positive cocci (enterococci and staphylococci) accounted for between 11.2% and 17.0% of isolates per annum (mean 14.8%) (P>0.5) (Table 1).
- Of the 6 antimicrobial agents tested against isolates of *E. coli*, a trend of decreasing susceptibility was observed for trimethoprim-sulfamethoxazole (P=0.040), cephalexin (P=0.0004), amoxicillin-clavulanate (P<0.0001), and ciprofloxacin (P=0.0009) (Table 2).
- Current (2016) *in vitro* susceptibilities of urinary isolates of *E. coli* to SXT (77.7%), cephalexin (88.5%), amoxicillin-clavulanate (79.1%), and ciprofloxacin (79.9%) appear compromised in comparison to nitrofurantoin (97.8%) and fosfomycin (99.3%).
- Annual ESBL rates among isolates of *E. coli* varied widely from 0.8% in 2007 to 10.1% in 2016 (Table 3).
- Annual AmpC rates among isolates of *E. coli* varied widely from 3.2% in 2008 to 0.7% in 2016 (Table 3).
- Percent susceptible to fosfomycin was ≥10% higher than to nitrofurantoin against ESBL-positive isolates (96.1% of ESBL-positive isolates were susceptible to fosfomycin) and MDR isolates (93.5% of MDR isolates were susceptible to fosfomycin).

Conclusions

- *E. coli* is the most common bacterial pathogen isolated from urine specimens of Canadian outpatients when culture is performed.
- The annual prevalence of *Enterobacteriaceae*, *P. aeruginosa*, and Gram-positive cocci among urine specimens from Canadian outpatients was consistent from 2007 to 2016.
- Fosfomycin (99.3% of isolates susceptible) and nitrofurantoin (97.3% of isolates susceptible) were the most active antimicrobial agents *in vitro* against *E. coli* isolated from Canadian outpatients from 2007 to 2016.
- *In vitro* susceptibility of urine isolates of *E. coli* to trimethoprim-sulfamethoxazole was <80% for 8 of the 10 years between 2007 and 2016.

Acknowledgements

The CANWARD study is supported in part by the University of Manitoba, Winnipeg Health Sciences Centre, National Microbiology Laboratory, and Paladin Labs.

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