Journal of Microbiology, Immunology and Infection (2013) 46, 202—209

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Changing antibiotic susceptibilities of community-acquired uropathogens in Greece, 2005—2010

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Received 16 February 2012; received in revised form 13 April 2012; accepted 23 May 2012

KEYWORDS
Adults;
Antibiotic resistance;
Community-acquired;
ESBL;
Urinary tract infections

Purpose: The purpose of this study was to determine the distribution and changes in the antibiotic susceptibilities of uropathogens isolated from adults with community-acquired urinary tract infections (CA-UTIs) in Crete, Greece, over a 6-year period.
Methods: This study was performed with isolates from outpatients with UTIs, collected between 2005 and 2010. Isolates were identified by standard methods and antimicrobial susceptibility testing was performed using the disk diffusion method and the VITEK2 is an automated system used for identification and antimicrobial susceptibility testing of microorganisms (BioMerieux). To identify changes in susceptibility patterns, we compared results of the period 2005—2007 to those of the period 2008—2010. We also compared the antibiotic susceptibilities of isolates between males and females.
Results: A total of 4011 community-acquired uropathogens were isolated during the period of 2005—2010. *Escherichia coli* was the most common organism and responsible for 68.9% of CA-UTIs, followed by *Proteus mirabilis* (6.8%), *Klebsiella pneumoniae* (6.4%) and enterococci (6%). A significant increase in resistance of *E coli* isolates was noted for β-lactams, monobactams, aminoglycosides, quinolones, and cotrimoxazole. The reverse trend was evident for nitrofurantoin. Higher resistance rates of community-acquired *E coli* and non-*E coli* Enterobacteriaceae were noted in males for ampicillin, amoxicillin plus clavulanic acid, cephalosporins, aminoglycosides, and quinolones. No significant sex differences were noted in the antibiotic susceptibility patterns of enterococci.
Conclusion: There is a concerning trend for increasing resistance among *E coli* and non-*E coli* Enterobacteriaceae responsible for CA-UTIs in Crete in recent years likely due to the

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http://dx.doi.org/10.1016/j.jmii.2012.05.012

inappropriate use of broad spectrum antibiotics, as a substitute for precise diagnostics and/or to increase the chances of therapeutic success.

Introduction

To optimize the use of empirical antibacterial therapy for community acquired urinary tract infections (CA-UTIs), physicians should know the etiology and susceptibility patterns of urinary pathogens in their community.

Most CA-UTIs reflect episodes of acute, uncomplicated cystitis. The Infectious Diseases Society of America (IDSA) guidelines for the treatment of acute uncomplicated cystitis in women recommend the use of a 3-day course of cotrimoxazole as empiric first-line therapy except in communities with resistance rates exceeding 10%—20% to cotrimoxazole among uropathogens.[1] Although the relationship between antibiotic consumption and resistance is complex and some studies show no significant change in antimicrobial susceptibility over time,[2] increased antibiotic use and inappropriate use of newer broad spectrum antibiotics due to the fear of therapeutic failure with older agents, selects for resistant organisms, and antibiotic resistance is increasing among community-acquired urinary pathogens worldwide.[3—5]

The University Hospital of Heraklion is the only tertiary hospital in the island of Crete, Greece, and serves a population of more than 700,000 people. In this study, we describe the *in vitro* antimicrobial susceptibility patterns of community-acquired uropathogens that were isolated in the microbiology laboratory of this hospital over the period January 2005 to December 2010.

Materials and methods

Patients

The patients of this study were adult (age >14 years) outpatients of both sexes diagnosed and treated for CA-UTIs in one of the several outpatient clinics of the University Hospital of Heraklion. A UTI was considered as community-acquired if the patient had not received intravenous therapy or specialized wound care, had not received hemodialysis treatment or antineoplastic chemotherapy within the 30 days prior to infection, was not hospitalized in an acute care center the last 90 days before diagnosis of UTI, and did not reside in a nursing home or long-term care facility.[6] Patients with urinary catheters were excluded, since by definition they were considered as having healthcare-associated or nosocomial UTIs. All urine samples were collected in the emergency room or in one of the outpatient clinics of the hospital. Duplicate positive urine cultures, i.e., cultures from the same episode of UTI were excluded.

Laboratory methods

Quantitative urine cultures were performed with standard techniques using Columbia blood and MacConkey agar plates (BioMérieux, Marcy l' Etoile, France).[7] Plates were incubated for 18—24 hours at 36°C. Isolate identification was done by standard biochemical methods, the API system, and the VITEK2 automated system (BioMérieux). Antimicrobial susceptibility testing was performed using the disk diffusion method and the VITEK2 automated system.

The following antibiotics were tested against Gram-negative isolates: ampicillin, amoxicillin plus clavulanic acid (CA), ticarcillin, ticarcillin plus CA, piperacillin, piperacillin/tazobactam, cephalothin, cefoxitin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, aztreonam, imipenem, tobramycin, amikacin, gentamicin, netilmicin, tetracycline, colistin, cotrimoxazole, nitrofurantoin, nalidixic acid, pefloxacin, ofloxacin, norfloxacin, and ciprofloxacin. Double-disk synergy test was used for preliminary classification of the isolates as extended-spectrum β-lactamase (ESBL) producers. The synergistic activity of CA with both ceftazidime and cefotaxime was confirmed by means of E-test special strips (AB Biodisk, Solna, Sweden) containing ceftazidime/ceftazidime plus CA and cefotaxime/cefotaxime plus CA.[8]

The following antibiotics were tested against enterococci: Ampicillin, ampicillin plus sulbactam, gentamicin [high level (HL) resistance], tetracycline, nitrofurantoin, ciprofloxacin, vancomycin, and teicoplanin. Quality control strains used for antimicrobial susceptibility testing included *E coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *K pneumoniae* ATCC 700603 (ESBL producer), and *Enterococcus faecalis* ATCC 29212. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria.[8]

Statistical analysis

The proportion of resistant organisms was calculated by dividing the number of urinary isolates resistant to each antibiotic by the number of organisms that were tested against that antimicrobial agent. Intermediately resistant and resistant organisms were grouped together. To test for changes in the antibiotic susceptibilities of uropathogens over time, Fisher's exact test was used to compare the antibiotic susceptibilities of *E coli*, non-*E coli* Enterobacteriaceae, and *Enterococcus* spp. between the first (1/2005-12/2007) and second half of the study period (1/2008-12/2010), and between males and females. All tests were two-tailed and statistical significance was set at p values < 0.05. Statistical analysis was performed by Graphpad Prism software (version 4, La Jolla, CA, USA).

Results

A total of 4011 uropathogens were isolated during the period of January 2005 to December 2010 from patients with CA-UTIs. The distribution of urinary pathogens by

Table 1 Distribution of community-acquired uropathogens by study year (2005–2010)

	2005	2006	2007	2008	2009	2010	2005–2010
Escherichia coli	393 (70.6%)	468 (68.9%)	430 (70.6%)	400 (63.4%)	537 (71.6%)	534 (68%)	2762 (68.9%)
Proteus mirabilis	34 (6.1%)	51 (7.5%)	34 (5.6%)	44 (7%)	57 (7.6%)	53 (6.8%)	273 (6.8%)
Proteus vulgaris	—	1 (0.2%)	—	1 (0.2%)	—	—	2 (0.05%)
Proteus penneri	—	1 (0.2%)	1 (0.2%)	—	—	—	2 (0.05%)
Klebsiella pneumoniae	21 (3.8%)	32 (4.7%)	32 (5.2%)	47 (7.4%)	46 (6.1%)	75 (9.6%)	253 (6.3%)
Klebsiella oxytoca	2 (0.4%)	5 (0.7%)	3 (0.5%)	7 (1.1%)	2 (0.3%)	4 (0.5%)	23 (0.6%)
Enterobacter spp.	12 (2.1%)	12 (1.8%)	14 (2.3%)	10 (1.5%)	8 (1.1%)	13 (1.7%)	69 (1.7%)
Citrobacter spp.	7 (1.2%)	7 (1%)	12 (2%)	14 (2.2%)	9 (1.2%)	15 (1.9%)	64 (1.6%)
Morganella morganii	2 (0.4%)	2 (0.3%)	—	—	4 (0.5%)	3 (0.4%)	11 (0.3%)
Serratia spp.	2 (0.4%)	—	2 (0.3%)	—	1 (0.1%)	4 (0.5%)	9 (0.2%)
Salmonella spp.	—	2 (0.3%)	—	—	—	1 (0.1%)	3 (0.07%)
Pseudomonas aeruginosa	18 (3.2%)	15 (2.2%)	10 (1.6%)	19 (3%)	16 (2.1%)	11 (1.4%)	89 (2.2%)
Other gram-negative nonfermenters	2 (0.4%)	3 (0.4%)	2 (0.3%)	3 (0.5%)	5 (0.7%)	1 (0.1%)	16 (0.4%)
Enterococcus faecalis	31 (5.6%)	42 (6.2%)	40 (6.5%)	43 (6.8%)	35 (4.7%)	38 (4.8%)	229 (5.7%)
Enterococcus faecium	2 (0.4%)	2 (0.3%)	1 (0.2%)	1 (0.2%)	4 (0.5%)	3 (0.4%)	13 (0.3%)
Streptococcus agalactiae	6 (1%)	13 (1.9%)	6 (1%)	17 (2.7%)	11 (1.5%)	12 (1.5%)	65 (1.6%)
Streptococcus pyogenes	—	1 (0.2%)	1 (0.2%)	1 (0.2%)	—	—	3 (0.07%)
Staphylococcus aureus	3 (0.5%)	2 (0.3%)	—	6 (1%)	1 (0.1%)	—	12 (0.3%)
Staphylococcus coag. negative	3 (0.5%)	5 (0.7%)	2 (0.3%)	4 (0.6%)	3 (0.4%)	3 (0.4%)	20 (0.5%)
Staphylococcus saprophyticus	19 (3.4%)	15 (2.2%)	18 (3%)	14 (2.2%)	11 (1.5%)	15 (1.9%)	92 (2.3%)
Corynebacterium spp.	—	—	1 (0.2%)	—	—	—	1 (0.02%)
Total	557	679	609	631	750	785	4011

study year is shown in Table 1. As expected, *E coli* was the most common organism and responsible for 68.9% of CA-UTIs in this study, followed by *P mirabilis* (6.8%), *K pneumoniae* (6.4%), and enterococci (6%, *E faecalis* 5.7%, *E faecium* 0.3%). As shown in Table 2, a significant increase in resistance of *E coli* isolates was noted between the two study periods for monobactams (aztreonam) and all β-lactam antibiotics tested except ticarcillin plus CA, a parenteral antibiotic that is available only for nosocomial use. The same was true for aminoglycosides with the exception of gentamicin in which the increased resistance rate over the second period did not reach statistical significance, and amikacin in which a borderline decrease in resistance in recent years was noted. Regarding quinolones, a significant increase in nonsusceptibility was noted during the second half of the study. Concerning cotrimoxazole, a commonly used antibiotic for uncomplicated CA-UTIs, a significant increase in resistance was noted from 20.2% during the years 2005–2007 to 24% during the period of 2008–2010 ($p = 0.0172$). On the other hand, the reverse trend was evident for nitrofurantoin, an infrequently used antibiotic in Greece, in which the resistance rate dropped for 9.1% to 4.2% between the first and the second half of the study period. The only three non-β-lactam antibiotics for which no significant change in resistance was noted between the two study periods were imipenem, tetracycline and colistin. Regarding the other Enterobacteriaceae, no significant increase in resistance to ampicillin and amoxicillin plus CA was noted between the first and second half of the study period, but the resistance rates were high in both periods. A significant increase in resistance of these uropathogens was also noted against the antipseudomonal penicillins ticarcillin, ticarcillin plus CA, piperacillin, and piperacillin/tazobactam. The same trend of increased resistance was noted for all tested cephalosporins except cephalothin, a first generation cephalosporin and cefoxitin, a cephamycin often grouped with the second-generation cephalosporins. A significant increase in resistance of the other Enterobacteriaceae was noted against all tested aminoglycosides, cotrimoxazole and all tested quinolones except nalidixic acid and pefloxacin in which the increased resistance rates over the period 2008–2010 did not reach statistical significance. As was noted with *E coli* isolates, a decrease in resistance rates to nitrofurantoin of non-*E coli* Enterobacteriaceae was noted over the years 2008–2010. Moreover, the other Enterobacteriaceae were significantly less susceptible to aztreonam and imipenem in recent years.

Regarding ESBL producing strains of *E coli,* a significant increase was noted between the first (22 of 1291, 1.7%) and second (51 of 1271, 3.5%) half of the study ($p = 0.0005$). About ESBL producing strains of *Klebsiella* spp., an increase was noted between the two periods from 5.3% (5 of 95) in 2005–2007 to 12.3% (23 of 181, $p = 0.00596$) in 2008–2010. Finally, three ESBL producing strains of *P mirabilis* were seen, all in the more recent years (two in 2009 and one in 2010).

Regarding enterococci (*E faecalis* and *E faecium*), no significant changes in antibiotic resistance were noted between the two study periods for ampicillin with or without sulbactam, tetracycline, nitrofurantoin, and ciprofloxacin. On the other hand, gentamicin-HL resistance was substantially lower in recent years, while enterococci resistant to glycopeptides were noted only during the period 2005–2007.

Table 2 Antibiotic susceptibilities by study year for *E coli*, non-*E coli* Enterobacteriaceae and Enterococci

E coli									
Antimicrobial agent	2005, $n = 393$ Resistant	2006,$n = 468$ Resistant	2007,$n = 430$ Resistant	2008,$n = 400$ Resistant	2009,$n = 537$ Resistant	2010,$n = 534$ Resistant	2005–07 (A), $n = 1291$ Resistant	2008–10 (B), $n = 1471$ Resistant	p value A vs. B
Ampicillin	143 (36.4%)	150 (32.1%)	173 (40.2%)	151 (37.7%)	252 (46.9%)	212 (39.7%)	466 (36.1%)	615 (41.8%)	0.0023
Amoxicillin plus CA	57 (14.5%)	60 (12.8%)	57 (13.3%)	60 (15%)	115 (21.4%)	81 (15.2%)	174 (13.5%)	256 (17.4%)	0.0045
Ticarcillin	138 (35.1%)	146 (31.2%)	167 (38.8%)	139 (34.7%)	227 (42.3%)	203 (38%)	451 (34.9%)	569 (38.7%)	0.0439
Ticarcillin plus CA	81 (20.6%)	59 (12.6%)	86 (20%)	65 (16.2%)	129 (24%)	80 (15%)	226 (17.5%)	274 (18.6%)	0.4578
Piperacillin	122 (31%)	146 (31.2%)	164 (38.1%)	135 (33.7%)	224 (41.7%)	193 (36.1%)	432 (33.5%)	552 (37.5%)	0.0285
Piperacillin/ tazobactam	17 (4.3%)	13 (2.8%)	15 (3.5%)	14 (3.5%)	41 (7.6%)	39 (7.3%)	45 (3.5%)	94 (6.4%)	0.0005
Cephalothin	146 (37.2%)	159 (34%)	160 (37.2%)	117 (29.2%)	188 (35%)	146 (27.3%)	465 (36%)	451 (30.7%)	0.0031
Cefoxitin	7 (1.8%)	15 (3.2%)	15 (3.5%)	11 (2.7%)	27 (5%)	35 (6.6%)	37 (2.9%)	73 (5%)	0.0061
Cefuroxime	11 (2.8%)	21 (4.5%)	19 (4.4%)	20 (5%)	41 (7.6%)	44 (8.2%)	51 (4%)	105 (7.1%)	0.0003
Cefotaxime	7 (1.8%)	9 (1.9%)	7 (1.6%)	7 (1.7%)	24 (4.5%)	21 (3.9%)	23 (1.8%)	52 (3.5%)	0.0047
Ceftriaxone	7 (1.8%)	9 (1.9%)	7 (1.6%)	7 (1.7%)	24 (4.5%)	21 (3.9%)	23 (1.8%)	52 (3.5%)	0.0047
Ceftazidime	7 (1.8%)	9 (1.9%)	7 (1.6%)	7 (1.7%)	24 (4.5%)	21 (3.9%)	23 (1.8%)	52 (3.5%)	0.0047
Cefepime	7 (1.8%)	9 (1.9%)	7 (1.6%)	7 (1.7%)	24 (4.5%)	21 (3.9%)	23 (1.8%)	52 (3.5%)	0.0047
Imipenem	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	0 (0%)	0 (0%)	1 (0.1%)	1
Aztreonam	7 (1.8%)	9 (1.9%)	7 (1.6%)	7 (1.7%)	24 (4.5%)	21 (3.9%)	23 (1.8%)	52 (3.5%)	0.0047
Tobramycin	11 (2.8%)	4 (0.8%)	16 (3.7%)	9 (2.3%)	31 (5.8%)	32 (6%)	31 (2.4%)	72 (4.9%)	0.0006
Amikacin	17 (4.3%)	21 (4.5%)	9 (2.1%)	5 (1.3%)	16 (3%)	14 (2.6%)	47 (3.6%)	35 (2.4%)	0.0562
Gentamicin	16 (4.1%)	13 (2.8%)	14 (3.2%)	10 (2.5%)	28 (5.2%)	27 (5.1%)	43 (3.3%)	65 (4.4%)	0.1682
Netilmicin	7 (1.8%)	3 (0.6%)	14 (3.2%)	9 (2.3%)	29 (5.4%)	26 (4.9%)	24 (1.9%)	64 (4.4%)	0.0002
Tetracycline	85 (21.6%)	116 (24.8%)	102 (23.7%)	101 (25.3%)	148 (27.6%)	116 (21.7%)	303 (23.5%)	365 (24.8%)	0.4230
Colistin	1 (0.2%)	1 (0.2%)	0 (0%)	1 (0.3%)	0 (0%)	1 (0.2%)	2 (0.2%)	2 (0.1%)	0.6279
Cotrimoxazole	78 (19.8%)	91 (19.4%)	92 (21.4%)	85 (21.3%)	143 (26.6%)	125 (23.4%)	261 (20.2%)	353 (24%)	0.0172
Nitrofurantoin	51 (13%)	46 (9.8%)	21 (4.9%)	17 (4.3%)	25 (4.7%)	20 (3.7%)	118 (9.1%)	62 (4.2%)	0.0001
Nalidixic acid	22 (5.6%)	39 (8.3%)	50 (11.6%)	34 (8.5%)	84 (15.6%)	67 (12.5%)	111 (8.6%)	185 (12.6%)	0.0008
Pefloxacin	20 (5.1%)	33 (7%)	40 (9.3%)	32 (8%)	68 (12.7%)	54 (10.1%)	93 (7.2%)	154 (10.5%)	0.0026
Ofloxacin	15 (3.8%)	26 (5.5%)	33 (7.7%)	24 (6%)	56 (10.4%)	50 (9.4%)	74 (5.7%)	130 (8.8%)	0.0021
Norfloxacin	17 (4.3%)	26 (5.5%)	33 (7.7%)	26 (6.5%)	57 (10.6%)	53 (9.9%)	76 (5.9%)	136 (9.2%)	0.0016
Ciprofloxacin	16 (4.1%)	25 (5.3%)	33 (7.7%)	24 (6%)	53 (9.9%)	52 (9.7%)	74 (5.7%)	129 (8.8%)	0.0027
Non-*E coli* Enterobacteriaceae									
Antimicrobial agent	2005, $n = 80$ Resistant	2006, $n = 114$ Resistant	2007, $n = 98$ Resistant	2008, $n = 122$ Resistant	2009, $n = 127$ Resistant	2010, $n = 168$ Resistant	2005–07 (A), $n = 292$ Resistant	2008–10 (B), $n = 417$ Resistant	p value A vs. B
Ampicillin	55 (68.7%)	81 (71.1%)	26 (26.5%)	94 (77%)	97 (76.4%)	35 (20.8%)	162 (55.5%)	226 (54.2%)	0.7594
Amoxicillin plus CA	24 (30%)	23 (20.2%)	22 (22.4%)	27 (22.1%)	43 (33.9%)	51 (30.4%)	69 (23.6%)	121 (29%)	0.1212
Ticarcillin	41 (51.2%)	67 (58.8%)	59 (60.2%)	83 (68%)	89 (70.1%)	115 (68.5%)	167 (57.2%)	287 (68.8%)	0.0019
Ticarcillin plus CA	11 (13.7%)	11 (9.6%)	13 (13.3%)	18 (14.7%)	29 (22.8%)	30 (17.9%)	35 (12%)	77 (18.5%)	0.0213
Piperacillin	34 (42.5%)	64 (56.1%)	55 (56.1%)	83 (68%)	86 (67.7%)	112 (66.7%)	153 (52.4%)	281 (67.4%)	0.0001

(*continued on next page*)

Table 2 (*continued*)

Non-*E coli* Enterobacteriaceae									
Antimicrobial agent	2005, $n = 80$ Resistant	2006, $n = 114$ Resistant	2007, $n = 98$ Resistant	2008, $n = 122$ Resistant	2009, $n = 127$ Resistant	2010, $n = 168$ Resistant	2005–07 (A), $n = 292$ Resistant	2008–10 (B), $n = 417$ Resistant	p value A vs. B
Piperacillin/ tazobactam	5 (6.2%)	8 (7%)	6 (6.1%)	14 (11.5%)	22 (17.3%)	24 (14.3%)	19 (6.5%)	60(14.4%)	0.0010
Cephalothin	26 (32.5%)	34 (29.8%)	28 (28.6%)	35 (28.7%)	43 (33.8%)	55 (32.7%)	88 (30.1%)	133 (31.9%)	0.6805
Cefoxitin	18 (22.5%)	18 (15.8%)	23 (23.5%)	22 (18%)	30 (23.6%)	44 (26.2%)	59 (20.2%)	96 (23%)	0.4064
Cefuroxime	12 (15%)	15 (13.2%)	14 (14.3%)	20 (16.4%)	29 (22.8%)	39 (23.2%)	41 (14%)	88 (21.1%)	0.0176
Cefotaxime	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	21 (16.5%)	20 (11.9%)	15 (5.1%)	52 (12.5%)	0.0010
Ceftriaxone	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	21 (16.5%)	20 (11.9%)	15 (5.1%)	52 (12.5%)	0.0010
Ceftazidime	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	21 (16.5%)	20 (11.9%)	15 (5.1%)	52 (12.5%)	0.0010
Cefepime	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	21 (16.5%)	20 (11.9%)	15 (5.1%)	52 (12.5%)	0.0010
Imipenem	0 (0%)	1 (0.9%)	0 (0%)	8 (6.6%)	7 (5.5%)	5 (3%)	1 (0.3%)	20 (4.8%)	0.0004
Aztreonam	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	21 (16.5%)	20 (11.9%)	15 (5.1%)	52 (12.5%)	0.0010
Tobramycin	4 (5%)	7 (6.2%)	7 (7.1%)	12 (9.8%)	19 (15%)	19 (11.3%)	18 (6.2%)	50 (12%)	0.0095
Amikacin	5 (6.2%)	3 (2.6%)	7 (7.1%)	10 (8.2%)	17 (13.4%)	19 (11.3%)	15 (5.1%)	46 (11%)	0.0062
Gentamicin	3 (3.7%)	7 (6.2%)	7 (7.1%)	11 (9%)	16 (12.6%)	15 (8.9%)	17 (5.8%)	42(10.1%)	0.0526
Netilmicin	3 (3.7%)	5 (4.4%)	7 (7.1%)	11 (9%)	19 (15%)	19 (11.3%)	15 (5.1%)	49(11.8%)	0.0022
Tetracycline	41 (51.2%)	67 (58.8%)	46 (46.9%)	62 (50.8%)	74 (58.3%)	76 (45.2%)	154(52.7%)	212(50.8%)	0.6471
Colistin	38 (47.5%)	54 (47.4%)	38 (38.8%)	48 (39.3%)	62 (48.8%)	62 (36.9%)	130 (44.5%)	172 (41.2%)	0.3968
Cotrimoxazole	9 (11.2%)	18 (15.8%)	13 (13.3%)	19 (15.6%)	31 (24.4%)	35 (20.8%)	40 (13.7%)	85 (20.4%)	0.0216
Nitrofurantoin	69 (86.2%)	96 (84.2%)	76 (77.6%)	36 (29.5%)	101 (79.5%)	131 (78%)	241 (82.5%)	268 (64.3%)	0.0001
Nalidixic acid	6 (7.5%)	16 (14%)	22 (22.4%)	21 (17.2%)	27 (21.3%)	29 (17.3%)	44 (15.1%)	77 (18.5%)	0.2649
Pefloxacin	6 (7.5%)	10 (8.8%)	12 (12.2%)	14 (11.5%)	22 (17.3%)	22 (13.1%)	28 (9.6%)	58 (13.9%)	0.1014
Ofloxacin	4 (5%)	9 (7.9%)	6 (6.1%)	10 (8.2%)	16 (12.6%)	21 (12.5%)	19 (6.5%)	47 (11.3%)	0.0355
Norfloxacin	4 (5%)	6 (5.3%)	7 (7.1%)	8 (6.6%)	16 (12.6%)	22 (13.1%)	17 (5.8%)	46 (11%)	0.0161
Ciprofloxacin	4 (5%)	10 (8.8%)	6 (6.1%)	11 (9%)	16 (12.6%)	21 (12.5%)	20 (6.8%)	48 (11.5%)	0.0389
Enterococcus spp.									
Antimicrobial agent	2005, $n = 33$ Resistant	2006, $n = 44$ Resistant	2007, $n = 41$ Resistant	2008, $n = 44$ Resistant	2009, $n = 39$ Resistant	2010, $n = 41$ Resistant	2005–07 (A), $n = 118$ Resistant	2008–10 (B), $n = 124$ Resistant	p value A vs. B
Ampicillin	7 (21.2%)	8 (18.2%)	6 (14.6%)	7 (15.9%)	12(30.8%)	9 (22%)	21 (17.8%)	28 (22.6%)	0.4242
Ampicillin plus sulbactam	7 (21.2%)	8 (18.2%)	6 (14.6%)	7 (15.9%)	12 (30.8%)	9 (22%)	21 (17.8%)	28 (22.6%)	0.4242
Gentamicin (HL resistance)	6 (18.2%)	12 (27.3%	19(46.3%	11 (25%)	7 (17.9%)	5(12.2%)	37 (31.4%)	23 (18.5%)	0.0255
Tetracycline	23 (69.7%)	27 (61.4%)	30 (73.2%)	35 (79.5%)	27 (69.2%)	27 (65.9%)	80 (67.8%)	89 (71.8%)	0.5755
Nitrofurantoin	2 (6%)	3 (6.8%)	2 (4.9%)	3 (6.8%)	3 (7.7%)	3 (7.3%)	7 (5.9%)	9 (7.3%)	0.7979
Ciprofloxacin	30 (90.9%)	39 (88.6%)	40 (97.6%)	44 (100%)	39 (100%)	37 (90.2%)	109 (92.4%)	120 (96.8%)	0.1592
Vancomycin	3 (9.1%)	0	1 (2.4%)	0	0	0	4 (3.4%)	0	0.0551
Teicoplanin	3 (9.1%)	0	1 (2.4%)	0	0	0	4 (3.4%)	0	0.0551

CA = clavulanic acid; HL = high level.

Regarding sex differences in the antibiotic susceptibilities, much higher resistance rates of community-acquired *E coli* were seen in males for ampicillin, amoxicillin plus CA, cephalosporins, aminoglycosides, and quinolones and borderline higher resistance rates for nitrofurantoin. Concerning the other non-*E coli* Enterobacteriaceae, the resistance rates were much higher in males for all tested antibiotics except nitrofurantoin. Finally, no significant sex differences were noted in the antibiotic susceptibility patterns of enterococci (Table 3).

Discussion

Although most antimicrobial susceptibility surveillance studies of urinary isolates focus on hospitalized patients,[9,10] it is becoming increasingly evident that non-susceptibility to commonly used antibiotics is a problem not only for hospitalized but also for outpatients with UTIs. The most remarkable finding of our study is that a significant increase in resistance rates of *E coli* and other Enterobacteriaceae has occurred in Crete in recent years against most of the commonly used antibiotics for CA-UTIs, with the remarkable exception of nitrofurantoin.

The successful treatment of CA-UTIs requires effective oral antibiotics that may be increasingly difficult to identify in case of resistant organisms. As clearly shown from our results, within a relatively short period of time, a substantial increase in the non-susceptibility rates of the Gram-negative community-acquired uropathogens to most antibiotics was noted. By contrast, susceptibility rates of enterococci appear to be relatively stable or decreasing in recent years.

Increasing resistance of *E coli*, the main causative pathogen of CA-UTIs to ampicillin and to a lesser extent cotrimoxazole has been demonstrated in several parts of the world in urinary tract isolates obtained from patients visiting general practitioners. In such areas, fluoroquinolones are frequently prescribed for CA-UTIs.[3] Unfortunately, as shown by our results, approximately 9%—10% of *E coli* and 11%—18.5% of other Enterobacteriaceae responsible for UTIs in outpatients of our island are already resistant to fluoroquinolones, a worrisome observation. Fluoroquinolone resistance is increasingly common in Southern Europe,[3] while it remains particularly low in Scandinavia. In a study from Norway among 7302 *E coli* UTI isolates tested, only 1.2% were fluoroquinolone-resistant.[11] In a previous study from Greece, the non-susceptibility rate of *E coli* to ciprofloxacin was 2.2%.[12] In another Greek study, the proportion of community-acquired urinary isolates resistant to norfloxacin was 17.8% for males and 5.5% for females.[10] Higher antibiotic resistance rates in uropathogens isolated from males have also been described by others, likely due to the typically nonthreatening and uncomplicated nature of UTIs in females.[3,13]

We did not formally study the reasons for this significant increase in resistance to fluoroquinolones, although this increase likely parallels the more widespread use of quinolones for community infections. Even though the Greek Drug Administration (EOF) requires culture-directed selection of fluoroquinolones for UTIs, it is clear that, in everyday clinical practice, many clinicians inappropriately circumvent these restrictions. Moreover, the ease of procuring antibiotics without a prescription in Greek pharmacies results in excessive and unreasonable use. CA-UTIs account for a substantial proportion of antibiotic consumption worldwide with important ecological and economic implications, while changes in antibiotic resistance rates have been observed to follow changes in prescription practices.[12] Since the completion of our study, we have intensified our efforts to educate the community physicians of our area about the proper use of antibiotics for CA-UTIs, emphasizing the need for culture-directed therapy and the need for restricted use of broad spectrum antibiotics, particularly quinolones.

The Surveillance Network Database of the United States conducted a survey of antimicrobial susceptibilities of 103,223 bacterial isolates recovered from urine samples of female outpatients.[5] In this sex-specific study, resistance of *E coli* isolates to cotrimoxazole varied significantly according to geographic region, ranging from 22% in the western United States to 10% in the Northeast. In that study, rates of resistance to ampicillin ranged from 30% to 40% among *E coli* and non-*E coli* isolates nationwide, and although there was significant geographic variability in resistance to ampicillin, this was unacceptably high, i.e., $>$ 25% throughout the country.[5] This was observed in our study as well among both *E coli* and non-*E coli* isolates, rendering ampicillin an inappropriate first line agent for patients with CA-UTIs.[5,14]

The frequency of antimicrobial resistance of *E coli* isolates shows a consistent geographical gradient, being greater in Southern Europe, particularly Spain and Portugal than in Northern Europe.[3] For example, in Granada, Spain 37% of *E coli* strains were resistant to amoxicillin plus CA, 33% to cotrimoxazole, and 22% to ciprofloxacin.[15] Similar results have been published from nine Spanish regions during 2002 and 2004. *E coli* was the main pathogen in both years (73% vs. 68.3%) followed by *P mirabilis* (7.2% vs. 6.4%) and *K pneumoniae* (5.4% vs. 5.2%). Amoxicillin (58.2%—58.7%), cotrimoxazole (30.8%—33.8%) and ciprofloxacin (22.6%—22.7%) showed the highest resistance rates, while fosfomycin (2.1%—2.8%) and nitrofurantoin (3.5%—5.7%) had the lowest resistance rates.[16] Unfortunately, we did not study the *in vitro* susceptibility of the uropathogens of our study to fosfomycin. In a French study of 1160 strains of community-acquired uropathogens, fosfomycin retained good activity against enterobacteriaceae.[17]

The *in vitro* susceptibility of our *E coli* isolates to nitrofurantoin was high (resistance 4.2% in recent years). Hence, nitrofurantoin appears to be an excellent treatment option for uncomplicated cystitis in our region and clearly superior to cotrimoxazole, a drug in which 20.2% of our *E coli* isolates were resistant to it. Moreover, nitrofurantoin was the most effective oral agent against enterococcal isolates. By contrast, nitrofurantoin was not a good treatment option for non-*E coli* Enterobacteriaceae, such as *P mirabilis, K pneumoniae,* or *P aeruginosa,* something previously shown by others.[18] Nitrofurantoin has been shown to be a better empirical therapy for primary UTIs in Sao Paulo, Brazil.[19] Regarding options for oral therapy for non- *E coli* Gram-negative uropathogens in our area, these were essentially limited to fluoroquinolones, even though $>$

Table 3 Antibiotic susceptibilities by sex and uropathogen for selected antimicrobials (*E coli*, other Enterobacteriaceae and *Enterococcus* spp.)

E coli	Males ($n = 601$)	Females ($n = 2161$)	p value
Antibiotics	Resistant (%)	Resistant (%)	
Ampicillin	276 (45.9)	805 (37.3)	0.0001
Amoxicillin plus CA	132 (22)	298 (13.8)	< 0.0001
Cephalothin	219 (36.4)	697 (32.3)	0.0562
Cefoxitin	44 (7.3)	66 (3.1)	< 0.0001
Ceftriaxone	32 (5.3)	43 (2.1)	< 0.0001
Amikacin	29 (4.8)	53 (2.5)	0.004
Cotrimoxazole	136 (22.6)	478 (22.1)	0.7393
Nitrofurantoin	50 (8.3)	130 (6.1)	0.0494
Ciprofloxacin	89 (14.8)	114 (5.3)	< 0.0001
Other non-*E coli* Enterobacteriaceae	Males ($n = 212$)	Females ($n = 496$)	p value
Antibiotics	Resistant (%)	Resistant (%)	
Ampicillin	137 (64.6)	251 (50.6)	0.0007
Amoxicillin plus CA	73 (34.4)	117 (23.6)	0.004
Cephalothin	85 (40.1)	136 (27.4)	0.001
Cefoxitin	63 (29.7)	92 (18.5)	0.0014
Ceftriaxone	33 (15.6)	34 (6.9)	0.0006
Amikacin	33 (15.6)	28 (5.6)	< 0.0001
Cotrimoxazole	56 (26.4)	69 (13.9)	0.0001
Nitrofurantoin	147 (69.3)	362 (72.9)	0.3614
Ciprofloxacin	35 (16.5)	33 (6.7)	0.0001
Enterococcus spp.	Males ($n = 101$)	Females ($n = 141$)	p value
Antibiotics	Resistant (%)	Resistant (%)	
Ampicillin	24 (23.8)	26 (18.4)	0.3369
Ampicillin plus sulbactam	24 (23.8)	26 (18.4)	0.3369
Gentamicin HL	29 (28.7)	31 (22)	0.2906

CA = clavulanic acid; HL = high level.

11% of these isolates were resistant to this class of antibiotics in recent years.

The ECO·SENS II study determined the antimicrobial susceptibility of community-acquired *E coli* urinary isolates in unselected women aged 18—65 years over the years 2007—2008 and compared the results with those obtained in the ECO·SENS I study (1999—2000).[20] Antimicrobial susceptibility testing of 150—200 *E coli* isolates per country to 14 antimicrobials was performed by disk diffusion using EUCAST breakpoints. With some exception, resistance to cefadroxil (representative of oral cephalosporins), nitrofurantoin, fosfomycin, gentamicin and third-generation cephalosporins was $< 2\%$. Resistance levels were higher for amoxicillin plus CA (2%—8.9%) and ciprofloxacin (0.5%—7.6%) and much higher to ampicillin (21.2%—34.0%), and cotrimoxazole (14.4%—18.2%). Resistance to quinolones (nalidixic acid from 4.3% to 10.2%, ciprofloxacin from 1.1% to 3.9%) and trimethoprim (from 13.3% to 16.7%) increased between the ECO·SENS I and ECO·SENS II studies.[20]

ESBLs are lactamases that confer bacterial resistance to β-lactam antibiotics and aztreonam.[6] We noted a significant increase in ESBL producing strains of *E coli* and *Klebsiella* spp. in recent years. ESBL production substantially complicates the treatment of CA-UTIs, ever since it severely limits the available therapeutic options. In a study from Turkey, 20.2% of *E coli* isolates produced ESBL.[13] ESBL production among UTI pathogens in the community has been described in Kuwait as well,[21] and for the first time in the recent ECO·SENS II study.[20] UTIs due to ESBL-producing *E coli* are emerging, even in countries with low antibiotic use like Switzerland.[6]

Our study is limited by the fact that we did not collect antibiotic resistance data by age groups, e.g., patients 15—35, 35—55, or > 55 years of age, since previous studies have shown higher resistance rates in younger individuals.[10] Moreover, it is a single center study; hence our results may not be applicable to other areas of Crete. However, due to the tertiary nature of our hospital, we believe that our results are representative of the recent, true antimicrobial susceptibilities of community-acquired uropathogens in Crete, the largest Greek island. Finally, we did not study the antibiotic prescription patterns for CA-UTIs in our area, because the lack of automation in many of the local pharmacies makes such a study almost impossible to execute. Hence, we cannot prove our hypothesis that the increasing resistance among Enterobacteriaceae responsible for CA-UTIs in Crete in recent years is the result of inappropriate prescription practices, such as the use of broad-spectrum antibiotics including quinolones.

In conclusion, there is increasing resistance of *E coli* and non-*E coli* community-acquired uropathogens in Crete in recent years. This is concerning because in addition to limiting the available therapeutic options, it has serious ecologic and financial consequences. The latter are particularly important nowadays that Greece is facing tremendous financial instability. Appropriate diagnostics and optimized antibacterial therapy are vital in order to limit this escalating antibacterial resistance. To this extent, continuous surveillance of antimicrobial susceptibility at the local, national and international levels remains important for CA-UTIs.

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