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Original

Antimicrobial susceptibility of clinical *Escherichia coli* isolates from uncomplicated cystitis in women over a 1-year period in Spain

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SUMMARY

High usage of antibiotics in Spain has led to an increase in resistance in urinary Escherichia coli *isolates in different geographic regions. The problem of resistance in urinary* E. coli *in Spain was investigated by gathering a large number of isolates from 20 different sites nationwide over a 1-year period from November 2003 to October 2004 in a large population of women. The objectives of this study were to assess the resistance to the antibiotics most commonly prescribed for community-acquired urinary tract infections (UTIs), according to age and different geographical areas of Spain, and to evaluate the potential association between geographical differences in quinolone consumption and resistance to* E. coli*. A total of 2,292 valid* E. coli *strains from female outpatients were isolated and sent to a single central reference laboratory for confirmation and susceptibility testing. Of these, 2,230 isolates were available for the age analysis. A two-sided* χ^2 *test was used to identify differences in resistance between age groups. Antibiotic units per province were purchased from IMS and consumption was expressed in units per 1,000 people per year. Univariate correlation (Pearson coefficient) between resistance to ciprofloxacin and quinolone consumption was calculated using a two-sided* p*-value. Resistance shown by* E. coli *was more common to ampicillin (52.1%) and cotrimoxazole (26%), followed by quinolones (18%), whereas resistance to amoxicillin-clavulanic acid, cefuroxime-axetil and fosfomycin was less than 3%. In the subgroup of women aged >65 years, resistance to ciprofloxacin was 29% compared to 13% for the subgroup of women <65 years (*$p<0.001$*). For these same subgroups, resistance rates were 32% vs. 23% for cotrimoxazole (*$p<0.001$*) and 56% vs. 50% for ampicillin (*$p=0.02$*), respectively. Statistically significant correlations were found between consumption of quinolones and* E. coli *resistance to ciprofloxacin (*$r=0.5$*;* $p=0.025$*). Resistance of* E. coli *isolates to quinolones varied significantly according to geographical areas, ranging from a high of 16.5% and 16.6% in the southern and eastern regions of Spain, respectively, to a low of 8% in the north in women aged <65 years. Additionally, the susceptibility to quinolones of* E. coli *isolates recovered from women aged >65 years was significantly lower across all regions of Spain than that of isolates recovered from younger women. Fosfomycin, amoxicillin/clavulanic acid and cefuroxime-axetil are the most suitable antibiotics for empirical treatment in Spain given the high 18% and 26% resistance rates to quinolones and cotrimoxazole, respectively. Higher resistance rates to ciprofloxacin were associated with being aged 65 years and over. These data need to be considered when recommending empirical therapy for acute cystitis.*

Key words: Urinary tract infections - *Escherichia coli* - Resistance

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Sensibilidad a los antimicrobianos de aislamientos de Escherichia coli *de mujeres con cistitis no complicada durante un periodo de un año en España*

RESUMEN

La frecuente utilización de antibióticos en España ha permitido la aparición de resistencias en las cepas de E. coli *urinarias aisladas en distintas regiones geográficas. Nosotros hemos analizado el problema de las resistencias de* E. coli *urinaria en España recogiendo un gran número de muestras en 20 centros distintos de todo el país durante un periodo de un año (noviembre de 2003 a octubre de 2004), procedentes de una gran población de mujeres. Los objetivos de este estudio fueron valorar las resistencias a los antibióticos que con más frecuencia se prescriben como tratamiento de las infecciones de vías urinarias adquiridas en la comunidad, así como según la edad de las pacientes y las distintas regiones geográficas de España, y valorar una posible asociación entre las diferencias geográficas en la utilización de quinolonas y las resistencias de* E. coli. *Se aislaron en total 2292 cepas de* E. coli *válidas de mujeres en régimen ambulatorio y se remitieron a un único laboratorio de referencia central para la confirmación del diagnóstico y la realización de las pruebas de sensibilidad. De todas estas muestras, 2230 estuvieron disponibles para el análisis por edad. Se realizó una prueba de* χ^2 *de dos colas para analizar las diferencias de las resistencias entre los grupos de edad. La información sobre unidades de antibióticos en cada provincia se obtuvo del IMS y el consumo se expresó en unidades por mil personas-año. Se realizó una correlación univariable (coeficiente de Pearson) entre la resistencia al ciprofloxacino y el consumo de quinolonas usando un valor de p bilateral. Las resistencias que mostró* E. coli *fueron principalmente frente a ampicilina (52,1%), cotrimoxazol (26%) y después quinolonas (18%), mientras que las resistencias a amoxicilina-ácido clavulánico, cefuroxima axetilo y fosfomicina fueron inferiores al 3%. En el grupo de mujeres mayores de 65 años, las resistencias al ciprofloxacino alcanzaron el 29%, lo que contrasta con el 13% en las de menor edad (*p *<0.001). En el caso del cotrimoxazol estos valores fueron del 32% y el 23% (*p *<0.001), y para ampicilina del 56% y el 50% (*p *= 0.02). Se encontró una correlación estadísticamente significativa entre el consumo de quinolonas y la resistencia de* E. coli *al ciprofloxacino (r = 0.5;* p *= 0.025). Las resistencias a las quinolonas de los aislamientos de* E. coli *variaron de forma significativa en función de las regiones geográficas, oscilando entre una elevada frecuencia, del 16,5% y 16,6%, en las regiones sur y este de España, hasta otras bajas como el 8% descrito en la zona norte del país en mujeres menores de 65 años. Además, la sensibilidad a las quinolonas de las cepas de* E. coli *aisladas en mujeres mayores de 65 años fue significativamente menor en todas las regiones de España en comparación con las aisladas de mujeres más jóvenes. Fosfomicina, amoxicilina-ácido clavulánico y cefuroxima axetilo son los antibióticos más adecuados para el tratamiento empírico en España, dadas las elevadas frecuencias de resistencia a las quinolonas y el cotrimoxazol, del 18% y el 26%, respectivamente. La edad mayor de 65 años se asoció a una frecuencia de resistencias al ciprofloxacino superior a la observada en mujeres más jóvenes. Esta información se debe tener en cuenta a la hora de recomendar un tratamiento empírico para la cistitis aguda.*

Palabras clave: Infecciones de vías urinarias - *Escherichia coli* - Resistencias

INTRODUCTION

Urinary tract infection (UTI) is currently one of the most common diseases encountered in medical practice and encompasses a wide range of clinical entities. A lower urinary tract infection (LUTI) or acute cystitis consists of a symptomatic inflammation of the urinary bladder due to infection, and in more than 95% of cases it is monomicrobial (1). Community-acquired uncomplicated cystitis is caused by *Escherichia coli* in approximately 90% of cases and, less commonly, by other *Enterobacteriaceae,* such as *Klebsiella* spp. and *Proteus* spp. (2, 3). In young women, *Staphylococcus saprophyticus* can also be an etiological agent. In the hospitalized patient with a complicated UTI, urethral catheter and/or previous antibiotic treatment, *E. coli* remains the most common cause in around 40% of isolates, however the incidence of other species detected is increasing (*e.g.,* other members of the *Enterobacteriaceae* family, enterococci, *Pseudomonas aeruginosa*, *Candida* spp.), and in certain wards, such as intensive care units (ICUs), *Acinetobacter* spp. and other multiresistant bacteria (1). UTI in women is a common problem in primary care settings, accounting for approximately seven million ambulatory care visits each year in the United States (4). Approximately one in three women will require antimicrobial treatment for a UTI before age 24, and 40-50% of women will have a UTI during their lifetime (5).

The current management of acute uncomplicated cystitis is usually empirical, without using a urine culture or susceptibility testing to guide therapy. This is based on the narrow and predictable spectrum of etiological agents that cause this kind of infection and their susceptibility patterns (2). However, as with many community-acquired infections, antimicrobial resistance to different antimicrobial families with differing degrees of intensity and in different geographical regions, is increasing in countries like Spain, due to the spread and high level of usage of antibiotics. Knowledge about such antimicrobial resistance rates to the corresponding families of *E. coli* is of the utmost importance when recommending the most suitable antibiotic treatment.

While clear evidence exists of the potential risk for transmission of resistant *E. coli* resulting from animal sources being fed antibiotics as growth promoters (6, 7), recent studies indicate the overuse of antibiotics to explain the abnormally elevated resistance rates to certain drugs in Spain over time (8).

This study aimed to investigate the problem of resistance in urinary *E. coli* in Spain by gathering a large collection of isolates from many different sites across the country over a 1-year period, calculating resistance rates, and establishing whether or not the potential differences found could be explained, at least in part, by differences in regional antibiotic consumption. In addition, the study aimed to establish the associations between age and more common resistance to antibiotypes among urine isolates of *E. coli.*

MATERIALS AND METHODS

Data collection

The data presented were obtained from a national surveillance program that collected the antimicrobial susceptibility data of 2,292 *E. coli* strains from clinical microbiology laboratories at 20 hospitals in Spain. Data from the laboratories enrolled in the program were sent to a single central reference laboratory for confirmation and susceptibility testing.

In order to limit the analyses to episodes of community-acquired UTI in women, the database was searched for isolates recovered from urine samples obtained from women aged 18- 65 years and elderly females (>65 years) in an outpatient setting from November 2003 to October 2004. The gold standard for a diagnosis of a UTI was a positive culture with more than $\geq 10^5$ CFU/ml of the single organism of *E. coli.* Repeat isolates of *E. coli* from the same patient were ignored. Urine specimen sources other than clean catch (catheterized urine) were excluded from the analysis.

E. coli susceptibility testing

Susceptibility to ampicillin, amoxicillin/clavulanic acid, cefuroxime-axetil, norfloxacin, ciprofloxacin, cotrimoxazole, nitrofurantoin and fosfomycin was determined using a dilution in Mueller-Hinton agar following standard procedures defined by the Clinical and Laboratory Standards Institute [CLSI, formerly the National Committee for Clinical Laboratory Standards (NCCLS)], 2003. Isolates were classified as susceptible (S), intermediately resistant (I) or resistant (R) according to the following respective minimum inhibitory concentration (MIC) breakpoints (mg/l): ampicillin ≤8, 16, ≥32 mg/l; amoxicillin/clavulanic acid ≤8/4, 16/8, ≥32/16 mg/l; cefuroxime-axetil ≤4, 8-16, ≥32 mg/l; norfloxacin ≤4, 8, ≥16 mg/l; ciprofloxacin ≤1, 2, ≥4 mg/l; cotrimoxazole ≤2/38, ≥4/76 mg/l; nitrofurantoin ≤32, 64, ≥128 mg/l; fosfomycin ≤64, 128, ≥256 mg/l, respectively. Reference strains *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used for protocol quality control.

Centers

Twenty microbiology laboratories corresponding to regional and university hospitals were selected across Spain. In order to analyze geographical resistance, the centers were located in four geographical areas (north, center south and east). The northern region incorporated five hospitals, the center six, the south four, and the east five (Fig. 1).

Statistical methods

A two-sided χ^2 test was used to test differences in resistance for age groups and antibiotype of resistance. Population figures at January 1, 2004 were retrieved from the website of the Spanish National Institute for Statistics (www.ine.es). Antibiotic units per province were purchased from International Marketing Services (IMS) and consumption was expressed in units per 1,000 people per year. Univariate correlation (Pearson coefficient) between resistance to ciprofloxacin and quinolone consumption was calculated using a two-sided *p*-value.

Local resistance in each of the four geographical areas was obtained by dividing the number of resistant isolates by the total number of strains collected in each geographical area and their 95% confidence intervals (95% CIs) were calculated by the binomial method.

RESULTS

Antibiotic resistance in *E. coli* isolates

Over a 12-month period, a total of 2,292 valid *E. coli* strains were isolated and sent to the central reference laboratory for confirmation and susceptibility testing. Total susceptibility results on the basis of NCCLS interpretative categories are shown in Table 1.

Resistance exhibited by *E. coli* was more common to ampicillin (52.1%) and cotrimoxazole (26%), followed by quinolones (18%). Amoxicillin-clavulanic acid, cefuroxime-axetil and fosfomycin had the lowest resistance rates for the *E. coli* strains (Table 1).

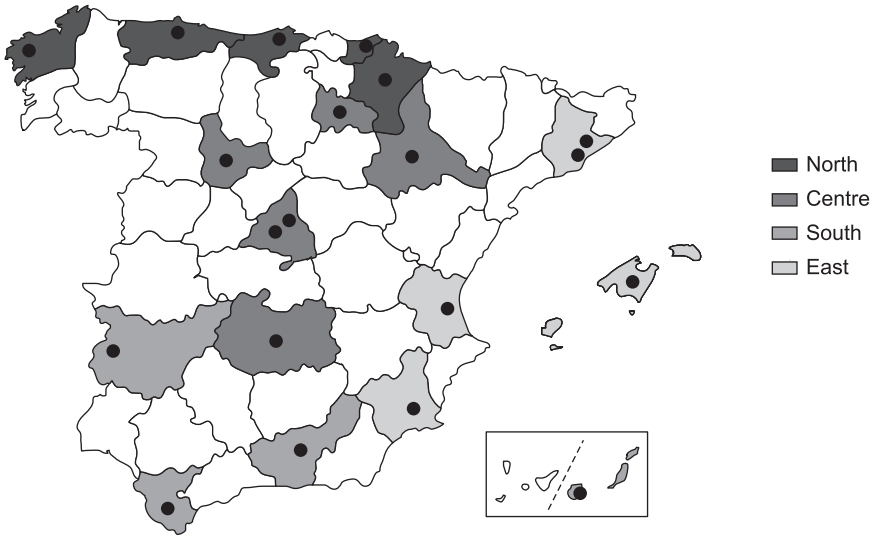

Figure 1. Map of Spain divided into four geographic areas (north, centre, south and east).

Influence of age on antibiotic resistance

From the total of 2,292 *E. coli* strains isolated, those from female outpatients of unknown age were excluded (1). The remaining 2,230 were available for the age analyses. Two-thirds of the isolates obtained in this study came primarily from women aged 18-65 years (67.2%).

In the subgroup of women aged older than 65, resistance to ciprofloxacin was 29% compared to 13% for women below that age (OR=2.8; 95% CI: 2.2-3.5; $p<0.001$). For cotrimoxazole rates were 32% *vs.* 23% (OR=1.6; 95% CI: 1.3-2.0; $p<0.001$), and for ampicillin 56% *vs.* 50% (OR=1.2; 95% CI: 1.0-1.5; $p=0.02$) (Table 2).

Influence of geographic variations on antibiotic resistance in relation to consumption

Quinolone consumption by site was calculated in units per 1,000 inhabitants per year (UIY). Large differences were noted across Spain, with antibiotic use being high in some areas, such as in the south, (155 UIY) and east (148 UIY), and low in other places, such as in the centre (87 UIY) (Fig. 2). The prevalence of resistance to ciprofloxacin averaged around 20%, varying from a minimum of 8.5% in the east to a maximum of 27.6% in the south.

Table 1. Susceptibility of the 2,292 valid isolates.

	NCCLS interpretative criteria; n (%)		
Antibiotic	Susceptible	Intermediate	Resistant
Ampicillin	1091 (47.6)	8 (0.3)	1193 (52.1)
Amoxicillin-clavulanic acid	2161 (94.3)	84 (3.7)	46 (2.0)
Cefuroxime-axetil	2188 (95.5)	48 (2.1)	55 (2.4)
Norfloxacin	1849 (80.7)	21 (0.9)	422 (18.4)
Ciprofloxacin	1866 (81.4)	18 (0.8)	418 (18.2)
Cotrimoxazole	1699 (74.1)	–	593 (25.9)
Nitrofurantoin	1526 (66.6)	623 (27.2)	143 (6.2)
Fosfomycin	2288 (99.8)	2 (0.1)	2 (0.1)

NCCLS: National Committee for Clinical Laboratory Standards.

Table 2. Resistance rates by age group; number of isolates resistant to the antibiotic (%).

Age	n	Ampicillin resistance (%)	Ciprofloxacin resistance (%)	Cotrimoxazole resistance (%)
≤65 years	1500	756 (50.4)	192 (12.8)	343 (22.9)
>65 years	730	406 (55.6)	210 (28.8)	236 (32.3)
Total	2230	1162 (52.1)	402 (18.0)	579 (26.0)
OR; [CI 95%]; *p*-value		OR=2.1; [1.0-1.5]; $p=0.02$	OR=2.8; [2.2-3.5]; $p<0.001$	OR=1.6; [1.3-2.0]; $p<0.001$

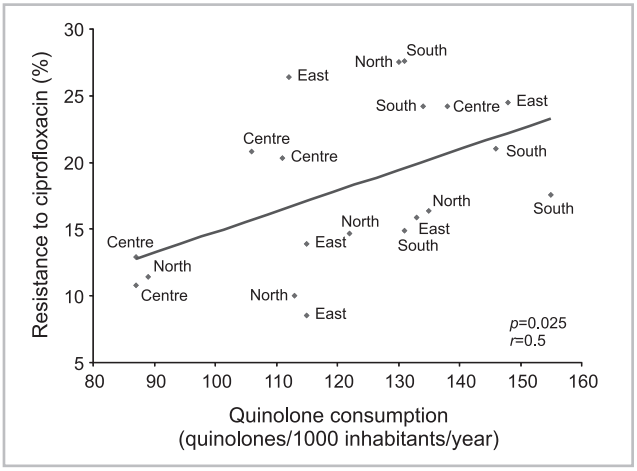

Figure 2. Provincial quinolone consumption and ciprofloxacin resistance in *E. coli.*

Total consumption of quinolone by site was associated with ciprofloxacin resistance, with a correlation coefficient of 0.5 ($p=0.025$).

The correlation between ciprofloxacin resistance by site and the corresponding consumption of quinolones is shown in Figure 1.

Resistance profiles in *E. coli* isolates

Among the 2,230 isolates available for the age analysis, 955 (42.8%) were susceptible to the three key antibiotics (ampicillin, cotrimoxazole and ciprofloxacin) (Table 3). Monoresistance to ampicillin, ciprofloxacin or cotrimoxazole was reported in 613 (27.5%) isolates: 23.6% to ampicillin, 2.1% to ciprofloxacin and 1.7% to cotrimoxazole, respectively (Table 4). Dual resistance was seen in 456 (20.4%) isolates: ampicillin was involved in up to 94% of these strains, ciprofloxacin in 32.7% and cotrimoxazole in 73.2%. Tripleresistance was observed in 206 (9.2%) isolates (Table 3).

Lower rates of resistance with increasing age were demonstrated for monoresistance to ampicillin (antibiotype D) (Table 4) with 25.6% of isolates from patients aged ≤65 years being resistant, compared with 19.6% for patients aged >65 years ($p=0.002$). Statistically significant rates of both dual resistance (antibiotype E and F) and triple-resistance (antibiotype H) were evident for patients aged older than 65 years ($p<0.001$)

The coresistance analyses are summarized in Table 4.

Table 3. Resistance to one or more antibiotics among 2,230 *E. coli* isolates tested with ampicillin, cotrimoxazole and ciprofloxacin.

Antibiotics to which isolates were resistant (n)	Total of isolates % (n)	Isolates resistant to % (n)		
		Ampicillin	Cotrimoxazole	Ciprofloxacin
0	42.8 (955)			
1	27.5 (613)	86 (527)	6.3 (39)	7.6 (47)
2	20.4 (456)	94 (429)	73.2 (334)	32.7 (149)
3	9.2 (206)	100 (206)	100 (206)	100 (206)

Table 4. Resistance profile to the three key antibiotics (ampicillin, ciprofloxacin and cotrimoxazole) among 2,230 isolates of *E. coli.*

Antibiotype	Ampicillin	Ciprofloxacin	Cotrimoxazole	Age		*p*-value	All (%)
				≤65 years (%)	>65 years (%)		
A	S	S	S	689 (45.9)	266 (36.4)	<0.001	955 (42.8)
B	S	S	R	25 (1.6)	14 (1.9)	NS	39 (1.7)
C	S	R	S	20 (1.3)	27 (3.7)	<0.001	47 (2.1)
D	R	S	S	384 (25.6)	143 (19.6)	0.002	527 (23.6)
E	S	R	R	10 (0.7)	17 (2.3)	<0.001	27 (1.2)
F	R	R	S	64 (4.2)	58 (7.9)	<0.001	122 (5.5)
G	R	S	R	210 (14)	97 (13.2)	0.007	307 (13.8)
H	R	R	R	98 (6.5)	108 (14.8)	<0.001	206 (9.2)
All				1500	730		2230

S: susceptible; R: resistant.

Resistance by age and geographic location

The prevalence of resistance among isolates of *E. coli* in relation to age and geographical areas is shown in Table 5.

Resistance to ampicillin and cotrimoxazole did not vary greatly by age group or within each of the four geographic areas analyzed (Table 5).

The highest rates of ciprofloxacin resistance were observed in the south (16.5%; 95% CI, 13.0-20.7) and in the east (16.5%; 95% CI, 12.3-21.5), while the lowest rates were seen in the north (8.1%; 95% CI, 5.5-11.5) in the subgroup of younger women. In the subgroup of women aged >65 years, quinolone resistance was three times more frequent among *E. coli* isolates in the north (ciprofloxacin: 25.1%; 95% CI, 19.8-31.0) than among those from women aged <65 years (ciprofloxacin: 8.1%; 95% CI, 5.5-11.5). Significant regional variations in resistance rates for quinolones in relation to age were observed in the remaining geographical areas (centre, south and east) (Table 5).

Table 5. Antimicrobial resistance (%; 95% CI) among 2,230 *E. coli* isolates by patient age and geographical areas of Spain.

	Ampicillin		Norfloxacin		Ciprofloxacin		Cotrimoxazole		Nitrofurantoin		Cefuroxime		Amoxicillin-clavulanic acid		Fosfomycin	
Antibiotic	≤65	>65	≤65	>65	≤65	>65	≤65	>65	≤65	>65	≤65	>65	≤65	>65	<65	>65
North	41.2% (36.0-46.5)	55.1% (48.7-61.5)	7.8% (5.3-11.1)	26.3% (20.9-32.3)	8.1% (5.5-11.5)	25.1% (19.8-31.0)	17.9% (14.1-21.3)	32.1% (26.3-38.4)	4.5% (2.6-7.2)	6.6% (3.8-10.5)	2% (0.8-4.0)	2.9% (1.2- 5.8)	2.8% (1.4-5.1)	2.1% (0.7-4.7)	0% (0-1)	0% (0-1.5)
Center	51.3% (46.8-55.8)	53.7% (47.4-59.9)	11.2% (8.6-14.3)	27.6% (22.3-33.5)	11.2% (8.6-14.3)	27.6% (22.3-33.5)	23.4% (19.7-27.4)	31.5% (25.9-37.6)	5.9% (4-8.4)	9.7% (6.4-14)	1.0% (0.3-2.4)	4.3% (2.2-7.5)	0.8% (0.2-2.1)	3.5% (1.6-6.5)	0.2% (0-1.1)	0.4% (0-2.1)
South	54% (47.9-60.1)	54.9% (46.0-63.5)	11.8% (8.2-16.2)	37.6% (29.3-46.4)	16.5% (12.3-21.5)	35.3% (27.3-44.1)	22.1% (17.3-27.5)	32.3% (27.5-41.0)	6.3% (3.7-9.8)	10.5% (5.9-17)	2.6% (1-5.2)	3.8% (1.2-8.6)	2.2% (0.8-4.7)	2.3% (0.5-6.5)	0% (0-1.3)	0% (0-2.7)
East	55.3% (50.1-60.3)	62.9% (52.5-72.5)	15% (11.6-19.0)	32% (22.9-42.2)	16.6% (13.0-20.7)	32% (22.9-42.2)	27.4% (22.9-32.1)	35.1% (25.6-45.4)	5.3% (3.2-8)	4.1% (1.1-10.2)	2.1 % (0.9-4.1)	4.1% (1.1-10.2)	1.6% (0.6-3.4)	2.1% (0.3-7.3)	0% (0-1)	0% (0-3.7)

DISCUSSION

This study provides current information regarding the main etiological agent *E. coli* that causes community-acquired UTI in women in the outpatient setting and its antimicrobial susceptibility patterns in relation to age and geographical areas on a national basis. In terms of consumption, antimicrobial agents for community-acquired UTI in Spain, updated to 2004 by IMS Ibérica, S.A. (Medical Data Index, MDI; Madrid, Spain), were ranked in the following order: quinolones (ciprofloxacin plus norfloxacin) (43.3%); fosfomycin (22.1%); amoxicillin-clavulanic acid (9.3%); cotrimoxazole (3.6%); cefuroxime-axetil (2.9%); and nitrofurantoin (1.1%).

By using this large national collection and by restricting the analyses to female outpatients within these two age groups, the population that is more suited to treatment with empirical therapy as recommended in the main international guidelines, was outlined (1, 9). The efficacy of such empirical therapy depends upon periodic assessment of antimicrobial resistance profiles. Surveillance of the susceptibility of *E. coli* to a range of antimicrobials is an essential part of monitoring and detecting any increase in resistance, and can aid clinicians in prescribing the most suitable antimicrobial agent for uncomplicated UTI in women.

Antibiotic resistance, which has traditionally been a problem only in nosocomial complicated UTI, is also now becoming a major risk in uncomplicated community-acquired UTIs. The high prevalence of resistance to ampicillin ob-

served in this study (52.1%) and reported as high as 30% and 50% in previously published studies conducted in Spain (10), USA (11), Canada (12) and western European countries (13), has precluded its use in empirical treatment.

Although the prevalence of *E. coli* resistant to cotrimoxazole varies considerably between different geographical areas, there is a persistent and significant decrease in the susceptibility to this antibiotic reported worldwide, including the USA where the resistance is now approaching 18-22% in some regions (11, 14). The Infectious Disease Society of America (IDSA) guidelines (1) recommend cotrimoxazole as a first-line agent for empirical therapy of uncomplicated cystitis only in the case that resistance is below 20%.

In the current study, as expected, *E. coli* exhibited a reduced susceptibility to cotrimoxazole (26%). This observation is in accordance with recent studies conducted in Spain (10) and Europe (13). It is interesting to note that some data have shown that the increase in cotrimoxazole resistance does correlate with poorer bacteriological and clinical outcomes (15, 16), even though this antibiotic can achieve high urinary concentrations.

Fluoroquinolone-resistant *E. coli* strains as a cause of uncomplicated UTI are being seen with increasing frequency in patients in large surveillance studies conducted over the past few years in Spain (10, 17-19). Recently, Alós *et al.* (18) carried out a Spanish surveillance study of susceptibility among urinary tract isolates collected from outpatients and demonstrated that the prevalence of ciprofloxacin resistance (22.8%) was extremely high for empirical treatment. An impressive decrease in the susceptibility of *E. coli* to fluoroquinolones has also been reported in Spain by Junquera *et al.* (17). They studied the variations in *E. coli* susceptibility patterns to commonly used antimicrobial agents in UTIs by stratifying isolates according to year and source. During the 8-year period, they noted a gradual decrease of susceptibility of this uropathogen to most of the antimicrobials tested but a terribly marked decrease for norfloxacin (from 85.1% in 1994 to 66.6% in 2001). Unsurprisingly, the data of the high rate of resistance to fluoroquinolones (18%) obtained in the present study are in line with these studies. Increasing fluoroquinolone resistance among *E. coli* has also been documented in studies conducted in other European countries (13, 20).

In the USA, fluoroquinolones have become the first line treatment for uncomplicated UTIs in many areas where resistance to cotrimoxazole is prevalent, but the widespread use of fluoroquinolones for such a common infection raised concerns regarding the possibility of accelerated development of resistance (1). Resistance to ciprofloxacin, although still relatively low, has shown a gradual increase as reported by several surveillance programmes over the past few years (21-24). Karlowsky *et al.* (21) noted that ciprofloxacin was the only agent that demonstrated a threefold increase in resistance from 1995 (0.7%) to 2001 (2.5%). A recent study (22) not only made an important observation on the differences between the USA and Canada in terms of resistance rates to commonly used agents for UTIs, but also noticed a higher ciprofloxacin resistance (9.7%) than reported in previous US surveillance studies (23, 24).

Given that the susceptibility to fosfomycin, amoxicillin-clavulanic acid and cefuroxime-axetil remained near to 95%, these three antibiotics are the most suitable options for empirical treatment of uncomplicated UTIs in Spain.

Efforts to control antibiotic prescribing are all based on the assumption that this will reduce, contain, or at least slow down the development and spread of bacterial resistance (25). The relationship between the development and extension of resistance and increased antibiotic use is well established (26-28), but the crucial issue is the direct demonstration of the reversibility of bacterial resistance following decreased antibiotic use. To support this link, there are some studies available (29-31), but unfortunately, none of these studies has investigated decreasing resistance after the use of quinolone antibiotics.

Several studies have investigated the correlation between quinolone prescribing and resistance in *E. coli*, in individual countries or within the regions of a country or in several countries of Europe (20, 32-34). In the study conducted by Goossens *et al.* (32), there are some striking findings related to the quinolone prescription that should be considered. First of all, total outpatient quinolone use in 26 countries in Europe in 2002 varied by a factor of 21.2 between the country with the highest (3.76 UIY in Italy) and the country with the lowest (0.17 UIY in Denmark) quinolone use. The total consumption of quinolones showed a strong positive correlation (r=0.74; p=0.0023) with the quinolone resistance in *E. coli.* In another study carried out in Slovenia, resistance of *E. coli* to ciprofloxacin in community isolates increased from 3.6 to 9.2% over 3 years, while the use of fluoroquinolones increased from 0.59 to 1.5 DDD per 1,000 inhabitants per day (33). In the Olomouc region of Czech Republic, Urbanek *et al.* (34) evaluated the dependence of *E. coli* resistance to fluoroquinolones on their use in the community setting and found a significant correlation (r=0.859; p=0.029) between the consumption of quinolones and the quinolone resistance in *E. coli*. The study conducted by Goettsch *et al.* (20) reported an increase of resistance to norfloxacin from 1.3% in 1989 to 5.8% in 1998 and the analysis of strata, classified by year, age and gender, showed an association between the consumption of fluoroquinolones and resistance to norfloxacin in *E. coli*

(p<0.001). In Spain, the number of fluoroquinolone prescriptions in the community setting have risen from 1.26 DDD per 1,000 inhabitants per day in 1987 to 2.4 DDD per 1,000 inhabitants per day in 2000 (35, 36).

Geographical differences of resistance to ciprofloxacin in *E. coli* have been shown clearly in this study, with a trend to being higher in those regions with higher rates of consumption (r=0.5; p=0.025) and although the most plausible reason for demonstrating the increasing number of *E. coli* resistant to quinolones in Spain is the overuse of this group of antibiotics, other causes cannot be disregarded. The clinical and epidemiological data of the patients, such as age, are of maximal importance when deciding the most suitable empirical treatment in uncomplicated UTIs. Other causes involved in quinolone resistance are the use of quinolones in animals for growth promotion and, to a lesser extent, in agriculture for plant protection, and in industry (6, 37), but the impact of resistance on animal health is often little recognized by general practitioners.

The influence of age has previously been shown to impact on antibiotic resistance rates in urinary isolates (10, 11, 14, 18, 22), mainly on the fluoroquinolone group. In the present study, *E. coli* resistance rates for ampicillin, cotrimoxazole and ciprofloxacin were higher in the elderly group (aged >65 years) compared with the other group of women (aged 18-65 years), reaching statistical significance for the three antimicrobials. Additionally, the prevalence of isolates resistant to two or three antibiotics was higher among isolates from women aged older than 65 years.

The current data showed a high percentage of coresistance (29.7%), among 2,230 isolates of *E. coli* available for the age analysis, either dual (20.4%) or tripleresistance (9.2%) (Table 3). This study has also shown that a ciprofloxacin-resistant antibiotype without resistance to other classes of antibiotics is very unusual (Table 3) and that the increasing rates of ciprofloxacin resistance become more common as the rates of concurrent resistance to ampicillin or cotrimoxazole increase.

This study found significant geographical differences in the susceptibility of quinolones for outpatient female UTI. Within Spain, the resistance rates of ciprofloxacin were highest in the south and in the east of the country and were lowest in the north (Table 5). Additionally, in the subgroup of women aged older than 65 years, significant regional variations in resistance rates for quinolones were observed in all geographical areas across Spain (Table 5). Clinicians should be aware of regional resistance rates and should take the age of the patient into consideration for better empirical treatment.

In conclusion, fosfomycin, amoxicillin-clavulanic acid and cefuroxime-axetil are the most suitable antibiotics for empirical treatment in Spain, given the high 18% and 26% resistance rates to quinolones and cotrimoxazole, respectively. Age over 65 years was associated with even higher resistance rates to ciprofloxacin (mean resistance: 29%) than below that age. In addition, rates of coresistance antibiotypes were more frequent in this subgroup of age. It is essential to be aware of local antimicrobial susceptibility patterns of urinary isolates in order to prescribe suitable antibiotics.

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