

## Original

# Quinolone resistance in female outpatient urinary tract isolates of *Escherichia coli*: Age-related differences

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### SUMMARY

During a 1-year period, from November 2003 to October 2004, urinary *Escherichia coli* isolates were collected from 20 clinical microbiology laboratories across Spain. The main objective was to assess the resistance of *E. coli* to the antimicrobials most commonly prescribed for community-acquired urinary tract infections depending on the patient's age. A total of 2,230 valid *E. coli* strains from female outpatients were isolated and sent to a single central reference laboratory for confirmation and susceptibility testing using an agar dilution method. A two-sided chi-squared test was used to assess the differences in resistance between age groups ( $\leq 65$  and  $> 65$  years). *E. coli* resistance was found to be more common to ampicillin (52.1%), cotrimoxazole (26%) and quinolones (18%), whereas resistance to amoxicillin-clavulanic acid, cefuroxime axetil and fosfomycin were below 3%. In women older than 65 years, resistance to ciprofloxacin reached up to 29% compared with 13% of those in the under 65 age group ( $p < 0.001$ ). For cotrimoxazole, rates were 32% vs. 23% ( $p < 0.001$ ) and for ampicillin 56% vs. 50% ( $p = 0.02$ ), respectively. It was concluded that fosfomycin, amoxicillin-clavulanic acid and cefuroxime axetil are the most suitable antimicrobials for empirical treatment in Spain given the high 18% and 26% resistance rates to quinolones and cotrimoxazole, respectively. Being older than 65 years of age was associated with higher resistance rates to ciprofloxacin (29%). These results should be considered when recommending empirical therapy for acute cystitis in women.

**Key words:** Resistance - Quinolones - *Escherichia coli* - Community-acquired urinary tract infection

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## Resistencia a las quinolonas en aislamientos de *Escherichia coli* procedentes de mujeres con cistitis aguda extrahospitalaria: diferencias en relación con la edad

### RESUMEN

Se recogieron a lo largo de un año (noviembre de 2003 a octubre de 2004) 2230 aislamientos de *Escherichia coli* de pacientes con infección urinaria adquirida en la comunidad procedentes de 20 laboratorios microbiológicos de toda España, que fueron enviados a un laboratorio central de referencia para realizar la confirmación del patógeno y las pruebas de sensibilidad antibacteriana, mediante el método de dilución en agar. El principal objetivo fue evaluar la resistencia a los antimicrobianos más prescritos en las cistitis agudas, en relación con la edad. Se utilizó el test de  $\chi^2$  bilateral para valorar las diferencias de la resistencia entre los dos grupos de edad ( $\leq 65$  años y  $\geq 65$  años). *E. coli* mostró una elevada resistencia a la ampicilina (52,1%), el cotrimoxazol (26%) y las quinolonas (18%), mientras que a amoxicilina-ácido clavulánico, cefuroxima-axetilo y fosfomicina fue inferior al 3%. En las mujeres mayores de 65 años, la resistencia al ciprofloxacino alcanzó el 29%, frente al 13% en las mujeres por debajo de esa edad ( $p < 0.001$ ). Para el cotrimoxazol los porcentajes fueron del 32% frente al 23% ( $p < 0.001$ ) y para la ampicilina del 56% frente al 50% ( $p = 0.02$ ). La fosfomicina, amoxicilina-ácido clavulánico y cefuroxima-axetilo son los antibióticos más adecuados para el tratamiento empírico de las cistitis agudas extrahospitalarias en España, debido a las elevadas tasas de resistencia a las quinolonas y al cotrimoxazol (18% y 26%, respectivamente). La edad  $> 65$  años se asoció con mayores tasas de resistencia al ciprofloxacino (29%). Estos resultados deberían tenerse en cuenta cuando se recomienda un tratamiento antimicrobiano empírico en mujeres con cistitis aguda comunitaria.

**Palabras clave:** Resistencia - Quinolonas - *Escherichia coli* - Cistitis agudas comunitarias

### INTRODUCTION

Acute uncomplicated urinary tract infections (UTIs) in women are a common problem in primary care settings, accounting for approximately 7 million outpatient visits each year in the United States (1). The highest incidence is found in young, sexually active women aged 20-40 years and in postmenopausal populations (2). Approximately one out of three women will require antimicrobial treatment for a UTI before age 24, and 40-50% of women will have a UTI during their lifetime (3). The microbiology of this syndrome is remarkably consistent, with *Escherichia coli* isolated in 80-85% of infections, *Staphylococcus saprophyticus* in 5-10%, and *Klebsiella pneumoniae* and *Proteus mirabilis* responsible for most of the remaining infections (1).

The current management of acute uncomplicated cystitis is usually empirical, without the use of a urine culture or susceptibility test to guide therapy. This statement is based on the narrow and predictable spectrum of etiologic agents that cause this kind of infection as well as on their susceptibility patterns (4). However, as with many community-acquired infections, antimicrobial resistance to different antimicrobial families in different geographical regions is increasing in countries like Spain due to the spread and high use of antimicrobial agents. For these two reasons, continuously updated surveillance of the antimicrobial susceptibility of *E. coli* would be helpful in guiding empirical treatment.

The main objective of this study was to conduct an age assessment of the resistance to the antimicrobial agents most commonly prescribed for *E. coli* community-acquired urinary tract infections.

### MATERIALS AND METHODS

#### Data collection

The data presented were obtained from a national survey of 20 clinical microbiology laboratories corresponding to regional and university hospitals throughout Spain. Isolates from the laboratories enrolled were sent to a single central reference laboratory for confirmation and susceptibility testing.

The isolates were obtained from urine samples from women aged 18 years and older in an outpatient setting and were collected between November 2003 and October 2004. During this period, each participating laboratory submitted the 10 first consecutive urine isolates of each month to reach a total of 120 valid samples, which were kept at  $-70$  °C. Samples were collected from women with a UTI who were treated at the emergency department, the outpatient unit of each of the participating hospitals, or the corresponding national health clinic. An *E. coli* isolate was considered valid if it came from women diagnosed with acute cystitis (based on signs and symptoms and/or significant presence of pyuria, leukocyte esterase or nitrites). In addition, a colony

count of  $\geq 10^5$  CFU/ml of *E. coli*, as a single organism in pure growth, was required. The only midstream urine samples were considered valid. In addition, catheterized urine samples were discarded, as were repeat isolates from a single patient. The isolates were sent to the central laboratory on a monthly basis, where isolates were cultured on MacConkey agar to confirm the etiology.

### *E. coli* susceptibility testing

Susceptibility to ampicillin, amoxicillin-clavulanic acid, cefuroxime axetil, norfloxacin, ciprofloxacin, cotrimoxazole, nitrofurantoin and fosfomicin were determined using an agar dilution method following standard procedures defined by the Clinical and Laboratory Standards Institute (CLSI) (5). Isolates were classified as susceptible (S), intermediately resistant (I) or resistant (R) according to the following respective MIC breakpoints (mg/l): ampicillin,  $\leq 8$ , 16,  $\geq 32$ ; amoxicillin-clavulanic acid,  $\leq 8/4$ , 16/8,  $\geq 32/16$ ; cefuroxime axetil,  $\leq 4$ , 8-16,  $\geq 32$ ; norfloxacin,  $\leq 4$ , 8,  $\geq 16$ ; ciprofloxacin,  $\leq 1$ , 2,  $\geq 4$ ; cotrimoxazole  $\leq 2/38$ ,  $\geq 4/76$ ; nitrofurantoin,  $\leq 32$ , 64,  $\geq 128$ ; fosfomicin,  $\leq 64$ , 128,  $\geq 256$ . Reference strains of *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as controls.

### Statistical methods

For the analysis of results, isolates were distributed into two groups according to the patients' age: 18-64 years, and  $\geq 65$  years. A two-sided chi-squared test was used to assess differences in resistance by age group and for resistance profiles in *E. coli* isolates. Differences in the prevalence of resistance between age groups were determined by calculation of odds ratios with 95% confidence intervals. A p-value  $< 0.05$  was considered statistically significant.

## RESULTS

### Antibiotic resistance in *E. coli* isolates

A total of 2400 isolates were collected, 2230 of which were considered valid. Total susceptibility for the 2230 *E. coli* isolates, based on CLSI interpretative categories, is shown in Table 1.

### Resistance profiles in *E. coli* isolates

Among the 2230 isolates, 955 (42.8%) were susceptible to the three key antimicrobial agents (ampicillin, cotrimoxazole and ciprofloxacin) (Table 2). Monoresistance to

**Table 1. Susceptibility of 2230 isolates according to CLSI criteria.**

Antimicrobial	%		
	Susceptible	Intermediate	Resistant
Ampicillin	47.5	0.4	52.1
Amoxicillin-clavulanic acid	94.3	3.7	2.0
Cefuroxime axetil	95.4	2.2	2.4
Norfloxacin	80.9	0.9	18.2
Ciprofloxacin	81.2	0.8	18.0
Cotrimoxazole	74.0	–	26.0
Nitrofurantoin	66.3	27.4	6.3
Fosfomicin	99.8	0.1	0.1

ampicillin, ciprofloxacin or cotrimoxazole was reported in 613 (27.5%) isolates. Dual resistance was seen in 456 (20.4%) isolates and triple resistance was observed in 206 (9.2%) isolates (Table 2).

### Influence of age on antibiotic resistance

From the total of 2230 *E. coli* strains, two-thirds of the isolates came primarily from women aged 18-65 (67.2%). In the subgroup of women older than 65 years, resistance to ciprofloxacin reached up to 29% compared to 13% in the 65 and under group (Table 3).

Both dual resistance (resistance to ciprofloxacin and cotrimoxazole or resistance to ampicillin and ciprofloxacin) and triple resistance (resistance to ampicillin, ciprofloxacin and cotrimoxazole) were statistically significant for patients older than 65 years ( $p < 0.001$ ). The co-resistance analyses are summarized in Table 4.

## Discussion

This study provides an update on *E. coli*, the main cause of community-acquired UTI in female outpatients, and its

**Table 2. Resistance to one or more antibiotics among 2230 *E. coli* isolates tested against ampicillin, cotrimoxazole and ciprofloxacin.**

No. of antibiotics to which isolates were resistant	Total % of isolates (No.)
0	42.8% (955)
1	27.5% (613)
2	20.4% (456)
3	9.2% (206)

**Table 3. Resistance rates to three key antimicrobial agents by age group. Number of isolates resistant to each antimicrobial (%).**

Age (years)	No.	Ampicillin	Ciprofloxacin	Cotrimoxazole
≤65	1500	756 (50.4)	192 (12.8)	343 (22.9)
>65	730	406 (55.6)	210 (28.8)	236 (32.3)
Total	2230	1162 (52.1)	402 (18.0)	579 (26.0)
OR		OR=1.2	OR=2.8	OR=1.6
[CI 95%]		[1.03-1.5]	[2.2-3.5]	[1.3-2.0]
p-value		p=0.02	p<0.001	p<0.001

antimicrobial susceptibility patterns in relation to age. Although the prevalence of *E. coli* resistant to cotrimoxazole varies considerably between different geographical areas, a persistent and significant decrease in the susceptibility to this antimicrobial has been reported worldwide, including the USA, where resistance now reaches 18-22% in some regions (6, 7). As expected, in the current study, *E. coli* showed reduced susceptibility to cotrimoxazole (26%). This observation is in accordance with recent studies conducted in other European countries (8, 9).

Fluoroquinolone-resistant *E. coli* strains causing uncomplicated UTI have been increasingly observed in large surveillance studies conducted over the past few years in Spain (8, 10, 11). Recently, Andreu *et al.* (8) conducted a susceptibility surveillance study of urinary tract isolates collected from outpatients and observed that the prevalence of ciprofloxacin resistance (22.8%) was extremely high, such that it should not be recommended for empirical treatment. Not surprisingly, the data for the high mean resistance rate to fluoroquinolones (18%) obtained in this study are in line with those studies. Increasing fluoroquinolone resistance among *E. coli* has also been documented in studies conducted in other European countries (9, 12). In the USA, resistance to ciprofloxacin, although still relatively

low, has been gradually increasing, as reported by several surveillance programs over the past years (13, 14).

Given that the susceptibility to fosfomycin, amoxicillin-clavulanic acid and cefuroxime axetil remained near 95%, these three antimicrobial agents are the most suitable options for empirical treatment of uncomplicated UTIs in Spain. These results are consistent with those of other studies conducted in other European countries (9, 10, 15).

Age has previously been shown to have an influence on antimicrobial resistance rates, mainly with the fluoroquinolone group (11, 14). One study (14) showed that ciprofloxacin and levofloxacin resistance rates increased with age, but no statistically significant differences were demonstrated when ampicillin and cotrimoxazole were compared according to age group. This observation was confirmed in the study carried out by Alós *et al.* (11) in which significant differences were found for the quinolones when the resistance of isolates from patients aged 50 years or older was compared with isolates from patients aged under 50, with no significant difference for any other antimicrobial. Contrary to those studies, in the current study, *E. coli* resistance rates for ampicillin, cotrimoxazole and ciprofloxacin were statistically significantly higher in the elder group (>65 years) compared with the other group of women (≤65 years). Ad-

**Table 4. Resistance profile to the three key antimicrobial agents (ampicillin, ciprofloxacin and cotrimoxazole) among 2230 *E. coli* isolates.**

Antibiotype	Ampicillin	Ciprofloxacin	Cotrimoxazole	≤65 years	>65 years	p-value	All (%)
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: susceptibility; R: resistance.

ditionally, the prevalence of isolates resistant to two or three antimicrobial agents was higher among isolates from women older than 65 years.

Furthermore, the data indicated a high percentage of co-resistance (29.6%) among clinical isolates of *E. coli*, either dual (20.4%) or triple resistance (9.2%). This study has also shown that a ciprofloxacin-resistant antibiotype without resistance to other classes of antimicrobial agents is very unusual and the increasing rates of resistance to ciprofloxacin become more common with the increasing rates of concurrent resistance to ampicillin or cotrimoxazole. Some previous studies have also reported co-resistance in urinary isolates of *E. coli* (7, 13, 16, 17). Zhanel *et al.* (16) showed a correlation between ampicillin and cotrimoxazole resistance, as well as reporting maximal rates of ampicillin (90%) and cotrimoxazole (100%) resistance for ciprofloxacin-resistant *E. coli*. Sahn *et al.* (7) demonstrated that, during 2000 in the USA, 7.1% of the 38,835 isolates were multidrug resistant (MDR) (resistant to three or more antimicrobials), and among the MDR isolates, 97.8% were resistant to ampicillin, 92.8% were resistant to cotrimoxazole and 38.8% were resistant to ciprofloxacin. The international survey, ECO-SENS Project (17), provided detailed information on the distribution of different phenotypes in 16 European countries and Canada. The results obtained have shown that a multiple-resistant phenotype involving fluoroquinolone resistance is present in most European countries and that this phenotype is selected not only by the use of quinolones but also by the use of ampicillin/amoxicillin and cotrimoxazole (17).

In conclusion, fosfomicin, amoxicillin-clavulanic acid and cefuroxime axetil are the most suitable antimicrobials for empirical treatment in Spain given the high resistance rates to quinolones and cotrimoxazole. The over-65 age group was associated with even higher resistance and co-resistance rates to ciprofloxacin and other antibiotics. In light of these results, quinolones should be used with caution in women over 65 for the treatment of uncomplicated UTI.

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