Original Article

High resistance prevalence towards ampicillin, co-trimoxazole and ciprofloxacin, among uropathogenic *Escherichia coli* isolates in Mexico City

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Abstract

Background : The prevalence of antimicrobial resistance among uropathogenic *E. coli* varies widely worldwide; to guide empirical therapy is necessary to have local, up-to-date susceptibility data. Methodology: We tested 907 isolates from patients in Mexico City by disk diffusion and further characterized ciprofloxacin, cephalosporin and nitrofurantoin resistant strains. Results: Isolates were mostly resistant to ampicillin (74%), trimethoprim-sulfamethoxazole (60.1%) and ciprofloxacin (32.6%). The most effective drug was netilmicin (5.1% resistant) and the most effective of oral drugs was nitrofurantoin (7.4% resistant). Sixty-percent of ciprofloxacin-resistant strains had minimal inhibitory concentrations of 125 µg/ml or higher, well beyond urinary concentrations at the end of the 12-hour inter-dose period for standard oral regimes. Extended-spectrum beta-lactamases were detected in 6% of strains, most of them from community-acquired infections. All strains resistant to nitrofurantoin carried a ~20 Kb plasmid, which when transformed into a susceptible recipient, conferred resistance to nitrofurantoin, ampicillin, sulfonamides, streptomycin, and partially protected against ciprofloxacin. Conclusions: Drugs considered of choice against uncomplicated urinary tract infections are facing high resistance prevalences and resistance determinants formerly seen only at hospitals are now among community strains. Treatment guidelines from developed countries might not reflect these local trends.

Key Words: uropathogenic *E. coli*, ampicillin, co-trimoxazole, ciprofloxacin, nitrofurantoin, antibiotic resistance

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Introduction

Resistance to some antibiotics among uropathogenic bacteria varies widely from one location to another and even along patients' age groups. Resistance towards fluoroquinolones in uropathogenic *E. coli*, for instance, ranges between less than 5% to more than 25% depending on the center in the US [1]. In an Argentinean study, resistance ranged from 2% in isolates from patients younger than 18 to 18%-26% in those older than 66 [2]. Moreover, resistance to some drugs varies also over time, mostly in increasing trends. However, for some antimicrobial agents, resistance seems to have reached a plateau, some at high prevalence levels, such as ampicillin (usually at 50% or higher), and some at low levels (nitrofurantoin; below 10%). These trends are often assumed to be the consequence of antibiotic abuse, although the biological features of resistance determinants, and the pharmacological attributes of drugs might also influence the prevalence of resistance. Here we report the resistance prevalence amongst 907 uropathogenic *E. coli* isolates from patients in Mexico City; Mexico, as many other developing countries, adds to the widespread medical abuse of antibiotics through the open availability of these drugs which leads to self-prescription [3]. Preliminary data on some of these isolates have been previously published [4].

Materials and Methods

Nine-hundred and seven strains were obtained from routine urine cultures, mostly from sexually-active or pregnant female outpatients. Isolates were identified using standard biochemical techniques and stored after isolation at -70° C in glycerol-containing medium until other tests were conducted. Susceptibility was tested by disk diffusion using commercially-available disks (BBL, Becton-Dickinson, Sparks, MD, USA) and following standard guidelines [5]. Antibiotics included were ampicillin (AM; 10 µg), amoxicillin-clavulanate (AMC; 20/10 µg), ceftazidime (CAZ; 30 µg), ceftizoxime (ZOX; 30 µg), ciprofloxacin (CIP; 5 µg), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), netilmicin (NET; 30 µg) and nitrofurantoin (FM; 300 µg). Further assessment of CIP minimal inhibitory

concentrations (MIC) was done by serial dilution in liquid Mueller-Hinton media (Fluka, Buchs, Switzerland). CAZ-ZOX-resistant strains were tested for extended-spectrum beta-lactamases (ESBL) by adding 30 µg of lithium clavulanate to CAZ and ZOX disks [6]. FM-resistant strains were screened for plasmids using an alkaline lysis mini-prep method (briefly, cells harvested by centrifugation from 1.5 ml of log-phase culture in liquid LB medium were treated sequentially with a glucose/Tris/EDTA solution, a NaOH/SDS solution and a potassium acetate solution, all at 4°C; DNA were recovered from supernatant after centrifugation, by precipitation with ethanol, then phenol-chloroform extracted, resuspended in TE buffer and separated by electrophoresis in 1% agarose gels [7]) and by pulsed-field gel electrophoresis (briefly, agarose plugs containing ~10^8 cells were treated sequentially with RNase/lysozyme/lauroyl sarcosinate, proteinase K, then washed and loaded into a 1% agarose gel, running for 11 hours at 180/120 V in a switch time ramp of 0.1-0.4 seconds, linear shape, in a FIGE Mapper device (Bio-Rad, Hercules, CA, USA) [8]).

Results and Discussion

Resistance prevalence is shown in Table 1; AM was the least effective drug *in vitro*, with 74% of isolates being resistant. NET was the most effective drug, with 5.1% resistant. FM was considered the most effective of the drugs that can be orally administered (7.4% resistant). These results are strinkingly similar to those reported recently by Sire *et al*. [9]. Mexican isolates were only less resistant than those from Senegal to AMC (27.2% *vs*. 67.5%), but more resistant to CIP (32.6% *vs*. 15.5%). Isolates of intermediate susceptibility to most drugs tested are very few or of minimal clinical relevance, as they can be considered fully susceptible to the concentrations reached clinically in the urine. For instance, NET-intermediate strains withstand no more than 32 µg/ml (while urine concentration is 40-50 µg/ml after 2 days of a 2.4 mg/kg/day treatment); CIP-intermediate strains withstand no more than 4 µg/ml (see next paragraph). Perhaps an exception is AMC, as only 18% to 38% of an oral dose of clavulanate is excreted unchanged in urine, and it is relatively unstable at 37°C [10].

According to CLSI breakpoints, a strain is deemed resistant to CIP when its MIC is 4 µg/ml or above [5]. However, as urinary concentrations of CIP can be around 50 µg/ml, 12 hours after taking an oral dose of 250 mg [10], that breakpoint could be irrelevant to urinary tract pathogens. Nevertheless, 62.8% of CIP-resistant strains had MICs ranging from 128 µg/ml or higher (Table 2), which would likely be enough to withstand concentrations attained clinically. Therefore, at least 20% of the strains studied here can be considered resistant to fluoroquinolones (taking CIP as representative of the group) at urinary concentrations. These results are much higher than those reported by others, where only 17% of CIP-resistant isolates had MICs ≥128 µg/ml [1], indicating that fluoroquinolone resistance among uropathogenic *E. coli* here is a much more serious threat than it is in the US. Whether this is the result of higher prescription/self-prescription rates in Mexico still remains to be established.

Table 1. Antibiotic susceptibility of uropathogenic *E. coli*.

Antibiotic	Resistant (%)	Intermediate (%)
AM	74.0	2.1
AMC	22.7	34.2
CAZ	8.1	1.8
ZOX	6.2	3.2
SXT	60.1	2.2
CIP	32.6	11.4
FM	7.4	4.4
NET	5.1	7.4

Resistance to CAZ in most (54/73) isolates was apparently caused by an ESBL, as susceptibility to CAZ in those strains was restored by clavulanate. Seven of them were not fully resistant to ZOX, but of intermediate susceptibility. The emergence of ESBLs in community-acquired infections has been reported recently. It is a dangerous trend that is appearing almost at the same time as community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) [11].

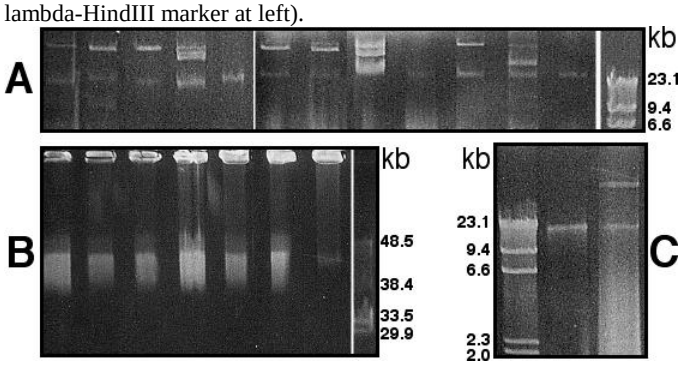
Table 2. Ciprofloxacin MICs (µg/ml) of CIP-resistant strains.

MIC	8	32	64	128	256	≥512
Strains	5	41	64	70	75	41

FM, introduced into clinical use more than 50 years ago, faces a low resistance rate. This is thought to be a consequence of the variety of effects it exerts upon the susceptible bacterial cell, against which a single resistance mechanism is difficult to mount. It might also be the result of a rather narrow range of clinical indications, which results in less usage. The clinical relevance of FM-resistance breakpoints might also be questionable: two out of three patients carrying FM-resistant uropathogens during a clinical trial were clinically and microbiologically cured with FM treatment [12]. FM-resistance might be associated with

patient age: while the average age of the patients sampled was 35.6 years old, the average age of those infected by a FM-resistant strain was 43.1 years old. This age-related effect was not detected with any of the other drugs tested (data not shown). Interestingly, all FM-resistant isolates carried a ~20 Kb plasmid (Figure 1); when this plasmid was transformed into strain DH5 [13] by electroporation [14], transformants gained resistance towards FM, AM, sulfonamide and streptomycin, according to disk diffusion assays, and decreased their susceptibility towards CIP, as inhibitory halos were reduced into the intermediate-susceptibility range (data not shown). Resistance towards FM is the result of diminished activity of nitroreductases, which activate the drug, either by mutations in encoding genes *nfsA* and *nfsB* [15], or by carrying a plasmid [16].

Figure 1. Large plasmids from NF-resistant strains.
A: Alkaline-lysis mini-preps from some of the NF-resistant strains, showing bands at and above 20-kb linear dsDNA (lambda-HindIII marker at right); B: PFGE of total undigested DNA from some NF-resistant strains (linear dsDNA size marker at right); C: One of such plasmids from mini-prep (right) and BamHI-digested (center; lambda-HindIII marker at left).



These results show a very high prevalence of resistance among uropathogenic *E. coli* towards the drugs of choice, such as co-trimoxazole and fluoroquinolones; CIP resistance towards the concentrations found in urine during therapy is not as high as indicated by standard breakpoints, but at 20% is still a cause of concern. Resistance determinants that were thought to be confined to hospital environments, such as ESBLs, were present in isolates from outpatients, which could jeopardize the clinical efficacy of third-generation cephalosporins even outside the hospitals. Resistance towards NF was of low prevalence, and apparently mediated by a large plasmid. Considering these *in vitro* data and NF's availability as an oral drug, nitrofurantoin should be considered as one of the drugs of choice against uncomplicated cystitis where multi-resistant uropathogenic *E. coli* are prevalent.

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