Original Article

Antimicrobial Resistance in Outpatient *Escherichia coli* Urinary Isolates in Dakar, Senegal

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Abstract
Background: Data regarding the evolution of antimicrobial resistance are needed to suggest appropriate empirical treatment of urinary tract infections (UTI) in developing countries. To assess the antimicrobial susceptibility of *Escherichia coli*, the predominant pathogen in community-acquired UTI, a prospective multicenter study was carried out in Dakar, Senegal.
Methodology: From February 2004 to October 2006, 1010 non-duplicate *E. coli* strains were collected from four centres. Antimicrobial susceptibility testing was performed using disk diffusion method according to the recommendations of the CA-SFM (2004).
Results: Most of the isolates were resistant to amoxicillin (73.1%), amoxicillin-clavulanic acid (67.5%), cephalothin (55.8%), and trimethoprim/sulfamethoxazole (68.1%). Extended spectrum beta-lactamase was detected in 38 strains. The overall resistance rates to nalidixic acid, norfloxacin and ciprofloxacin were 23.9%, 16.4% and 15.5%, respectively. Most of the strains were susceptible to gentamicin, nitrofurantoin and fosfomycin (respective susceptibility rates, 93.8%, 89.9%, and 99.3%). During this period, a significant decrease in sensitivity was observed for cephalothin, fluoroquinolones and trimethoprim/sulfamethoxazole (p<0.001).
Conclusions: These data suggest that trimethoprim/sulfamethoxazole may no longer be used as empirical treatment for community-acquired UTI in Dakar. In order to preserve the activity of fluoroquinolones for future years, alternatives such as fosfomycin or nitrofurantoin should be considered.
Key Words*: Escherichia coli*, urinary tract infection, antimicrobial resistance, outpatient, Senegal.

Introduction

Symptomatic urinary tract infection (UTI) is one of the most commonly diagnosed infections in communities, particularly in otherwise healthy young women. Several studies conducted in Europe and in the USA have reported a steady increase of the resistance rate of uropathogens to commonly prescribed antibiotics (amoxicillin, trimethoprim-sulfamethoxazole), reducing therapeutic possibilities [1-3]. In some countries, resistance to fluoroquinolones is also emerging [4]. In developing countries, the frequently irrational use of antibiotics predicts a worrisome increase in the prevalence of antimicrobial resistance.

Worldwide, *Escherichia coli* has been established as the most commonly isolated pathogen among organisms implicated in community-acquired UTI. In Senegal the same situation prevails [5]. Hence, a prospective three-year study was undertaken, from February 2004 to October 2006, to determine the susceptibility patterns of *E. coli* strains isolated from outpatients referred from four clinical laboratories located in Dakar, the capital of Senegal.

Materials and Methods
Patients

From February 2004 to October 2006, a total of 1010 *E. coli* strains isolated from the urine of patients with community-acquired urinary tract infections was collected from four clinical laboratories in Dakar. Two of these laboratories (Institut Pasteur, Bio24) are private institutions receiving patients referred by general practitioners. The two others are located inside hospitals, one being a major teaching hospital (Hôpital Aristide Le

Dantec) and the other a military hospital (Hôpital Principal). These hospital laboratories recruited subjects examined in the outpatients department with a suspected diagnosis of UTI. The patients attending the private laboratories, as well as the military hospital, belonged to upper and middle classes, while the majority of samples examined in the teaching hospital belonged to patients from the underprivileged classes. Pregnant women aged between 15 and 45 years were recorded. Patients were questioned about some specific conditions (i.e. hospitalization, antibiotic treatment, UTI and urinary catheterization) that could have occurred during the month preceding the onset of the symptoms. The nature of previously prescribed drugs was not included in the statistical analysis because very few patients were able to name the prior antibiotic used precisely.

Bacterial strains

Leukocyte count, isolation and identification of bacteria were conducted on site from urine samples by all the laboratories as part of their routine diagnostic. Only a pure culture of *E. coli* with a colony count of $\geq 10^5$ cfu/ml associated with pyuria (WBC $\geq 10^4$ / ml) was considered significant for inclusion. All *E. coli* isolates were collected for antimicrobial susceptibility testing by the coordinating laboratory located in Institut Pasteur. Species identification was checked by inoculation onto a culture medium allowing differentiation of main uropathogens (Chromagar orientation medium, Becton-Dickinson, Heidelberg, Germany). According to the manufacturer's instructions, indole-positive, pink-coloured colonies were confirmed as *E. coli*. In case of uncertainty, clear identification was performed using API 20E gallery (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Antibiotics' susceptibility was performed by disk diffusion method according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) [6]. Antibiotic disks were obtained from Bio-Rad laboratories (Marnes-la-Coquette, France) and strains were tested against the following antimicrobial agents (quantity of antibiotic per disk): amoxicillin (10 µg), amoxicillin-clavulanic acid (20 µg/10 µg), ticarcillin (75 µg), cephalothin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), nalidixic acid (30 µg),

norfloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (cotrimoxazole) (1.25 µg/23.75 µg), gentamicin (15 µg), nitrofurantoin (300 µg), and fosfomycin (50 µg). Extended spectrum beta-lactamase (ESBL) producing organisms were detected by the double-diffusion method looking for synergy images between amoxicillin-clavulanic acid and cefotaxime, or ceftazidime disks. Isolates producing ESBL were considered to be resistant to cefotaxime and ceftazidime according to the CA-SFM recommendations. Inhibition zone diameters were measured with an automated zone size reader (Osiris, Bio-Rad) and isolates were classified as susceptible, intermediate or resistant according to the annual report of the CA-SFM [6]. Intermediate and resistant strains were further grouped together in the resistant group for analysis. *E coli* ATCC 25922 was used as the control strain.

Statistical analysis

Statistical analysis was performed using STATA software version 6.0. Risk factors predicting *E. coli* resistance were estimated by univariate analysis and tested when appropriate by χ^2 or Fisher's exact test. Variables with P < 0.25 in the univariate analysis were then included in a multivariable logistic regression model. Variables with an adjusted odds ratio of P < 0.05 were considered as risk factors.

Results

One thousand and ten non-duplicate *E. coli* strains (561 from Institut Pasteur, 225 from Bio24, 177 from the teaching hospital laboratory, 47 from the military hospital laboratory), were collected during the study period. Seven hundred and seventy strains (76.3%) were isolated from women and 239 (23.7%) from men (sex status unknown for 1 patient). Patients were classified in three categories according to age: patients under 15 years (115;11.5%), patients aged from 15 to 50 years (635;63.6%) and patients over 50 years (249;24.9%), data was not available for 11 patients (1.1%). Among women aged from 15 to 45 years, 16.7% (84/504) were pregnant. The percentage of patients presenting potential risk factors for antimicrobial resistance was recorded as follows: hospitalization (4.6%, data reported for 940 patients), antibiotic exposure (16.4% of 971

patients), UTI (9.6% of 948 patients) and urinary catheterization (2.4% of 918 patients). Missing data were due to the difficulty in collecting oral information from some patients.

Resistance rates of *E. coli* strains to selected antimicrobial agents by year of study are presented in Table 1.

Table 1. Antimicrobial resistance of *E. coli* isolates from community acquired urinary tract infections by antibiotic and year of study.

Year (no. isolated)	No. of isolates resistant to antibiotics (%)			
	2004 (289)	2005 (449)	2006 (272)	Total (1010)
AMX	208 (72.0)	323 (71.9)	207 (76.1)	738 (73.1)
AMC	186 (64.4)	302 (67.3)	194 (71.3)	682 (67.5)
TIC	190 (65.7)	307 (68.4)	190 (69.9)	687 (68.0)
CF	134 (46.4)	254 (56.6)	176 (64.7)	564 (55.8)
CTX	8 (2.8)	13 (2.9)	14 (5.2)	35 (3.5)
CAZ	10 (3.5)	15 (3.3)	13 (4.8)	38 (3.8)
NA	58 (20.1)	110 (24.5)	73 (26.8)	241 (23.9)
NOR	31 (10.7)	72 (16.0)	63 (23.2)	166 (16.4)
CIP	28 (9.7)	69 (15.4)	60 (22.1)	157 (15.5)
SXT	169 (58.5)	323 (71.9)	196 (72.1)	688 (68.1)
GE	16 (5.5)	22 (4.9)	25 (9.2)	63 (6.2)
FUR	27 (9.5)	45 (10.0)	30 (11.0)	102 (10.1)
FOS	4 (1.4)	1 (0.2)	2 (0.7)	7 (0.7)

AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin; CF, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; NA, nalidixic acid; NOR, norfloxacin; CIP ciprofloxacin; SXT, trimethoprim/sulfamethoxazole (cotrimoxazole); GE, gentamicin; FUR, nitrofurantoin; FOS, fosfomycin.

The majority of the isolates were resistant to amoxicillin, amoxicillin-clavulanic acid, ticarcillin and first generation cephalosporins (respective overall resistance rates: 73.1%, 67.5%, 68% and 55.8%). Third generation cephalosporins (cefotaxime, ceftazidime) remained active in most cases (respective overall resistance rates: 3.5% and 3.8%). ESBL was detected in 38 strains (3.8%): 14 strains in 2004 (4.8%), 12 strains in 2005 (2.7%), and 12 strains in 2006 (4.4%). A high resistance rate was found for cotrimoxazole (68.1%). As for quinolones, 23.9% of the strains were resistant to nalidixic acid, 15.5% to ciprofloxacin and 16.4% to norfloxacin. Most of the

strains were susceptible to gentamicin (93.8% susceptible) and nitrofurantoin (89.9% susceptible). Almost all strains were susceptible to fosfomycin (99.3% susceptible).

During this study, a significant increase was observed for resistance to cephalothin (P<0.001), norfloxacin (P<0.001), ciprofloxacin (P<0.001) and cotrimoxazole (P<0.001). According to multivariate analysis (Table 2), age > 50 years was identified as a risk factor for resistance to nalidixic acid, norfloxacin and ciprofloxacin.

Table 2. Variables statistically related to antimicrobial resistance in the multivariate model.

Risk factor	Amoxicillin OR [95% CI]	P value	Cefotaxime OR [95% CI]	P value	Ceftazidime OR [95% CI]	P value	Gentamicin OR [95% CI]	P value
Sex								
Female			ref		Ref		ref	
Male			4.0* [1.8-8.9]	0.01	3.3* [1.6-7.1]	0.002	2.2* [1.2-4.1]	0.01
Prior antibiotherapy	1.8* [1.0-3.1]	0.03						

Risk factor	Nalidixic acid OR [95% CI]	P value	Norfloxacin OR [95% CI]	P value	Ciprofloxacin OR [95% CI]	P value	Cotrimoxazole OR [95% CI]	P value
Age category								
<15 years	ref		ref		Ref			
15-50 years	1.6 [0.9-3.0]	0.13	1.7 [0.8-3.9]	0.17	1.9 [0.8-4.4]	0.12		
> 50 years	4.2* [2.2-7.8]	<0.001	6.1* [2.8-13.4]	<0.001	6.9* [3.0-15.8]	<0.001		
Prior antibiotherapy	1.7* [1.0-2.7]	0.04	1.7 [1.0-3.0]	0.05	1.8* [1.1-3.2]	0.03	1.7* [1.1-2.5]	0.01

OR, odds ratio.

*Significant difference (P<0.05)

Male gender was significantly linked to a higher rate of resistance to gentamicin and third generation cephalosporins (cefotaxime, ceftazidime). Antibiotic exposure within the month before infection was significantly associated with resistance to amoxicillin, nalidixic acid, ciprofloxacin, cotrimoxazole and approached significant resistance to norfloxacin (P=0.05). Within the same period, previous hospitalization, prior UTI, and prior urinary catheterization were not considered in our model to be risk factors of antimicrobial resistance to any of the tested drugs.

Discussion

Antimicrobial susceptibility testing of uropathogens is known to overestimate rates of resistance because antibiograms are performed mostly if empirical treatment fails, or if patients have underlying factors [7]. In developing countries, this bias may be more present because many patients cannot afford medical expenses, so they undergo urine analysis only in case of repeated or complicated UTI. Nevertheless, this study confirms that levels of antimicrobial resistance of outpatient *E. coli* urinary isolates to aminopenicillins, cotrimoxazole and fluoroquinolones are higher in Dakar than those observed in Europe and North America [1,2]. This is consistent with recent data reported in other developing countries [8-11].

Previous data collected in our laboratory [5,12,13] confirm the steady decrease in susceptibility of *E. coli* strains isolated from community-acquired UTI to amoxicillin-clavulanic acid, cephalothin, quinolones, and cotrimoxazole since 1999. To date, cotrimoxazole is still recommended by Senegalese health authorities as the first line treatment for UTI in primary health care structures, which can explain the high level of resistance rate observed in this study. The significant increase of resistance to quinolones, and the high resistance rates reached in 2006, are worrisome and could be related to the easy access and the misuse of this class of antibiotics in Senegal [13]. Another factor could be the generalized use of fluoroquinolones in animal feed in the country (especially in poultry intensive breeding), and the subsequent transmission of resistant strains from animals to humans [14].

Accuracy of the risk factors analysis must be put into perspective because information was obtained directly from patients without checking their medical reports. Nevertheless, multivariate analysis highlighted some specific risk factors. Antibiotic exposure a month before infection was reported by multivariate analysis as a risk factor for resistance to amoxicillin, nalidixic acid, ciprofloxacin, and cotrimoxazole. This data partially confirms a previous study conducted in our laboratory, when antibiotic exposure within the previous six months was found associated with resistance to amoxicillin, amoxicillin-clavulanic acid and cotrimoxazole [13]. In our study, age over 50 years and male gender were found statistically significant with resistance to fluoroquinolones by univariate analysis (P<0.01). This result has been previously reported and could be related to the tendency for male or elderly patients to be infected by more resistant strains because they are more likely to present complicated urinary tract infections [15]. In our multivariate model, if age > 50 years remained independently associated with resistance to fluoroquinolones (P<0.001), male gender did not. In addition, a significant relationship has been found between male gender and resistance to third generation cephalosporins and gentamicin. To our knowledge, association to male gender has not been previously recorded for these antibiotics.

Aminopenicillins or cotrimoxazole should no longer be used as empirical treatments of UTI in Senegal. Alternatives must be recommended, especially for empirical treatments of uncomplicated UTI (cystitis) in women. Although most *E. coli* isolates were susceptible to nitrofurantoin, this antimicrobial agent has demonstrated poor *in vitro* activity against Enterobacteriaceae other than *E. coli* [16]. Moreover, this antibiotic does not penetrate into tissues and could not be used to treat infections with suspected tissue involvement (such as pyelonephritis). Generalized prescription of fluoroquinolones as empirical treatment for cystitis should be abandoned. This common practice facilitates the emergence of strains resistant to this class of antibiotics and promotes the emergence of multidrug-resistant strains in the community [17]. A substantial effort has to be made in Senegal to protect the activity of fluoroquinolones by controlling their prescription in human medicine and limiting (or even banning) their use in animal husbandry.

A previous study conducted in Dakar has shown that resistance rates of Enterobacteriaceae to fosfomycin-trometamol, a form of fosfomycin characterized by a high oral biodisponibility, was very low [18]. Our data about *E. coli* confirm these findings. Fosfomycin-trometamol has been proposed as a reliable empirical treatment for uncomplicated UTI because of its easy use (single dose), its good tolerance, and its efficacy [19]. Although *in vitro* resistance to fosfomycin is easily acquired by multiple one-step mutations, treatment failures are rare. A recent study suggests that the biological fitness of these resistant mutants is affected, so they are less likely to infect the bladder [20]. Unfortunately, this antibiotic is not yet available in Senegal. However, to preserve the efficacy of fluoroquinolones, we suggest that fosfomycin could be an option for empirical treatment of urinary tract infections in outpatients, and should be introduced into the national guidelines.

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