Original Article

Increasing prevalence of antimicrobial resistance among *Enterobacteriaceae* uropathogens in Bangui, Central African Republic

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Abstract

Background: Because of the previous high prevalence of resistance to usual antibiotics among uropathogens in Bangui, Central African Republic (CAR), a survey focused on *Enterobacteriaceae*, the most prevalent group responsible for urinary tract infections (UTIs), was conducted. The aim was to help health authorities revise antibiotic policies.

Methodology: We performed a retrospective analysis of all cases of confirmed UTIs due to *Enterobacteriaceae* in outpatients attending the Institut Pasteur de Bangui (IPB), CAR, between 2004 and 2006.

Results: During the study period, 560 (10.9% of urine submissions) UTIs were confirmed and 443 *Enterobacteriaceae* strains were isolated, representing 79% of the causative agents for UTIs. Among these 560 strains, *E. coli* was the most common, representing 64% of the isolates, followed by *K. pneumoniae* (10%) and other *Enterobacteriaceae* (5%). Extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* significantly increased from five (3.7%) to thirty-three (19.3%) between 2004 and 2006. A significantly increased resistance rate to nalidixic acid, ciprofloxacin and gentamicin was observed in ESBL-nonproducing *Enterobacteriaceae* over the study period.

Conclusions: Empiric treatment for UTIs in Bangui should be reconsidered and prudent use of antibiotics, particularly ciprofloxacin, is highly recommended. The recent spread of ESBL-producing *Enterobacteriaceae* in central African outpatients is striking and underlines the need for further studies.

Key words: urinary tract infection, extended-spectrum beta-lactamase, outpatients, Central Africa

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Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases diagnosed in outpatients and are frequently caused by *Enterobacteriaceae* strains. Most UTIs in developing countries are treated on an empirical basis; thus treatment should be based on available local data regarding the susceptibility of common pathogens to antibiotics. Unfortunately, most patients who can afford drugs are prone to self-treatment in the absence of any laboratory investigation. Therefore, an increase in antibiotic resistance is to be expected and it is important to determine the distribution of pathogens responsible for UTIs and their patterns of resistance to the main available antibiotics. In a previous survey, a high rate of resistance to commonly used antibiotics (amoxicillin, trimethoprim/sulfamethoxazole and quinolones) has already been described [1]. Such observations have also been made in other developing countries including Sudan [2], Madagascar [3], and Nicaragua [4]. In contrast, lower resistance rates to these antibiotics have been reported in a recent study of pregnant women from Tanzania [5].

The Central African Republic (CAR) is one of the poorest countries in the world and generic drugs are often the only affordable antibiotics. This study aimed to assess the evolution of resistance of *Enterobacteriaceae* strains isolated from UTIs in Bangui to the antibiotics available in the country and, consequently, to aid health authorities in revising the recommended first-line antibiotic treatments.

Materials and Methods

We conducted a retrospective study of all non-duplicate *Enterobacteriaceae* strains isolated from outpatients with suspected uncomplicated UTIs who attended the Institut Pasteur de Bangui (IPB) between January 2004 and December 2006. Urine samples were cultured on bromocresol purple agar plates (bioMérieux, Marcy l'Etoile, France). Only urine from patients with pyuria (>10 white blood cells/µL)

Table 1. Evolution of the resistance of *Enterobacteriaceae* isolated from UTIs in Bangui between 2004 and 2006.

	Percentage of isolates resistant to antibiotics (%)							
	All *Enterobacteriaceae*				ESBL-nonproducing strains			
Year (no. isolated)	2004 (136)	2005 (135)	2006 (171)	p	2004 (131)	2005 (123)	2006 (138)	P
Amoxicillin	89	86	90	NS	89	85	88	NS
Amoxicillin/clavulanic acid	52	62	66	0.04	50	58	60	NS
Cephalothin	30	46	49	<0.0001	27	40	40	NS
Cefotaxime	4	10	22	<0.0001	0	2	3	NS
Gentamicin	9	18	33	<0.0001	6	12	20	0.002
Nalidixic acid	21	31	50	<0.0001	19	24	38	0.001
Norfloxacin	18	25	45	<0.0001	16	19	33	0.002
Ciprofloxacin	16	25	44	<0.0001	15	19	31	0.003
Nitroxoline	76	82	89	0.01	75	82	87	0.05
Trimethoprim/sulfamethoxazole	88	84	86	NS	88	84	84	NS
Fosfomycin	3	3	2	NS	3	2	1	NS

NS: non significant

and substantial bacteriuria (> 1 x 10^5 cfu/mL) involving a single pathogen was included. *Enterobacteriaceae* strains were Gram stained and then identified with API 20E strips (bioMérieux). Susceptibility of these strains to antibiotics was determined by the disc diffusion method on Mueller-Hinton agar as recommended by the Comité de l'antibiogramme de la Société Française de Microbiologie (CA-SFM; Antibiogram Committee of the French Microbiology Society) [6]. The following antibiotics were tested: amoxicillin, amoxicillin/clavulanic acid, ticarcillin, cephalothin, cefoxitin, cefotaxime, ceftazidime, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, nitroxoline, and fosfomycin. Extended-spectrum beta-lactamase (ESBL) strains were systematically searched for by a double-diffusion method with cefotaxime or ceftazidime and amoxicillin/clavulanic acid as recommended [6]. Diameters of growth inhibition areas were measured with the automated Osiris system (Bio-Rad, Marnes la Coquette, France). *Enterobacteriaceae* strains were classified as susceptible, intermediate, or resistant according to the CA-SFM criteria. Intermediate and resistant strains were then grouped together and reclassified as resistant. *Escherichia coli* ATCC 25922 was used as a control strain.

To prove the increase in resistance of isolates with time, differences between proportions were tested using a Chi^2 for trends (STATA version 8.0; Stat corp., Texas) A *P*-value of < 0.05 was considered significant.

Results

From 2004 to 2006, bacterial pathogens were isolated from 560 of the 5,128 (10.9%) patients who submitted urine samples at the IPB. *Enterobacteriaceae* strains were cultured from 443 (79%) at a significant concentration, confirming UTIs: 357 (64%) *Escherichia coli*, 57 (10%) *Klebsiella pneumoniae*, 12 (2%) *Salmonella* spp. and 17 (3%) belong to other species (ten *Proteus* spp., three *Enterobacter* spp., four *Citrobacter* spp., one *Morganella morganii*). In 2004, 136 strains were isolated, 135 in 2005 and 171 in 2006. The distribution of strains among species did not vary significantly during this three-year period.

Among patients, the sex ratio was 1.5 with 267 female (60%) and 176 male (40%). Twenty-four percent of these 443 patients were under 15, 49% in the age group 15 to 45 and 27% over 45. There was also no significant difference in the distribution of species between age groups and genders.

Fifty ESBL-producing strains were identified in three years: 29 *E. coli*, 17 *K. pneumoniae*, 3 *Enterobacter* spp. and one *M. morganii*. An increasing prevalence of ESBL-producing *Enterobacteriaceae* in UTIs was noteworthy: five (3.7%) were detected in 2004, 12 (8.9%) in 2005 and 33 (19.3%) in 2006 ($p < 0.001$). These strains were as common in patients under 15 years old (22 strains) as in patients from 15 to 45 years old (19 strains).

Resistance to amoxicillin and trimethoprim/sulfamethoxazole was very high (> 85%) and stable over the three-year period. In contrast, only one to three percent of these strains

were resistant to fosfomycin. Resistance to gentamicin (9 to 33%), nalidixic acid (21 to 50%), ciprofloxacin (16 to 44%) and cefotaxime (4 to 22%) increased significantly between 2004 and 2006 (Table 1). These data directly relate to the significant increase in ESBL-producing strains. In addition to high-level resistance to beta-lactams, 46 of 50 ESBL-producing *Enterobacteriaceae* were also resistant to ciprofloxacin. Additionally, resistance to gentamicin, quinolones, and fluoroquinolone of ESBL-nonproducing strains also increased during this three-year period.

Discussion

Urinary tract infections are a common problem in general practice and are usually treated empirically. Empirical treatments should be based on local data regarding common pathogens and their susceptibility to available antibiotics. Patients attending the IPB are likely a selected population with more complicated clinical courses or underlying debilitating conditions and thus may be more likely to harbour a resistant bacterium. Difficulties in obtaining representative data about distribution and susceptibility patterns of uropathogens from laboratory data are well-documented [7]. Our study population presenting with UTI was selected mainly because it could afford the cost of urine examination As the collection of data and the laboratory methods were consistent throughout the study period, the evolution of the susceptibility over this three-year period should represent the general trend among urinary tract pathogens.

As reported previously in Bangui [1] and in other developing [2,8] as well as in developed countries [9-11], *E. coli* was the main pathogen responsible for UTIs, followed by *K. pneumoniae.*

According to multicentre studies conducted in Europe [9,11] and in North America [10], resistance to amoxicillin and trimethoprim/sulfametoxazole in UTIs from *E. coli* is no higher than 38% and 30%, respectively; however, there were important differences between neighbouring countries. A very high rate of resistance to these two antibiotics is observed in Bangui that can be explained by the high selective pressure exerted by an intensive use of inexpensive and easily administered drugs for many years; moreover, trimethoprim/sulfamethoxazole is commonly used to prevent HIV-associated opportunistic infections. Finally, the distribution of antimicrobial agents in pharmacies in the absence of any medical prescription and the sale of inexpensive drugs of uncertain origin in the parallel drug markets may contribute to these high resistance rates.

In this study, the increasing resistance of *E. coli* and *K. pneumoniae* to third-generation cephalosporin over three years is striking. This phenomenon is associated with the presence of ESBLs. The diffusion of a CTX-M-15 type beta-lactamase in clinical strains of *E. coli* from Bangui has recently been reported [12]. The number of bacteria presenting the same antibiogram profile as these ESBL-producing strains is increasing, even in patients with community-acquired infections who were observed from 2004 to 2006 [12]. This spread of ESBL-producing strains (particularly CTX-M enzymes) at the community level, causing disease or colonisation, has been previously reported [13]. It was not possible to identify risk factors associated with ESBL-producing strains because our study was retrospective; however, the very common use of generic ciprofloxacin for treatment of both in- and outpatients in Bangui may have contributed to a selection of these ESBL-producing strains, which are often multi-resistant, specifically to ciprofloxacin, trimethoprim/sulfamethoxazole and gentamicin. Thus, these strains are difficult to treat, particularly in resource-poor countries where the number of available drugs is limited.

Nevertheless, increasing resistance to fluoroquinolones and gentamicin are also observed for ESBL-nonproducing *Enterobacteriaceae*. For example, prevalence of resistance to ciprofloxacin, which was at10% in 2003 [1], went up to 31% in 2006 (table 1).

Our findings emphasize the need to reconsider the general use of antibiotics in CAR and highlight the lack of information about antibiotic use and drug resistance among the medical population. We strongly advise the prudent use of ciprofloxacin to prevent not only a further increase of resistance, but also a similar rapid occurrence of a very high level of resistance that was observed with trimethoprim/sulfamethoxazole and amoxicillin. A large survey of ESBL-producing clinical isolates will be conducted in Bangui to determine the main risk factors of the acquisition of such strains and to facilitate the planning of preventive actions, thus preventing the spread of these threatening pathogens.

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