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Original article

Diversity and antibiotic resistance of uropathogenic bacteria from Abidjan

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Abstract

Background: Urinary tract infections (UTI) are one of the major causes of prescribing and antibiotic consumption. In order to use the best antibiotic treatment for their patients, reliable and recent data about epidemiology and antibiotic resistance profile of uropathogenic bacteria must be available for clinicians. Therefore regular monitoring in each country is required.

Objectives: The aims of this study were to investigate the bacterial pathogenic diversity and antimicrobial resistance rates of uropathogenic bacteria at the Treichville Teaching Hospital (Abidjan, Ivory Coast) over a 12-year period (2000–2011) and also to contribute to the monitoring and the geographical adaptation of antibiotic therapy.

Materials and methods: A retrospective analysis of 12,175 urine samples over a 12-year period 2000–2011 at Treichville Teaching Hospital was carried out according to the routine protocol of urinalysis. The results were processed to obtain the profile prevalence of UTI, the rate of bacterial resistance to antibiotics, the trend of their evolution over time and the rate of multidrug resistance.

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Results: The presence of bacteria was detected in about 25% of samples in which 3071 bacterial germs belonging to 12 species were identified. *Escherichia coli* was the dominant species (28.7%) but much lower than observed in European countries (70–80%). Other main detected species were *Staphylococcus aureus* (17.4%), *Klebsiella pneumoniae* (14.9%) and *Enterobacter aerogenes* (10%). These genera were responsible of 71% of the UTI.
Resistance tests to antibiotics indicated very high rates of resistance to amoxicillin (78.9%), tetracyclin (76.4%), and trimethoprim/sulfamethoxazole (77.9%). Only a few molecules maintain their effectiveness such as cefotaxime and netilmicin which respectively exhibit 13.9% and 3.1% of bacterial resistance. However bacterial resistance is increasing over a time for all antibiotics except chloramphenicol.
Conclusions: The diversity of uropathogenic bacteria obtained appeared to be a characteristic of sub-Saharan African countries. Their resistances to different antibiotics were following a dramatic trend. Waiting to be confronted with therapeutic dead end with the advent of multi-resistant bacteria, identifying the region-specific causes is crucial to adapt antibiotic therapy.

Introduction

Urinary tract infections (UTI) are one of the major reasons for antibiotic prescribing [1]. Among the uropathogenic bacteria, *Escherichia coli* is predominant in both community and nosocomial UTI. However, the diversity of uropathogens is known to vary regionally [2]. Infections are gradually becoming more and more difficult to treat and may lead to therapeutic dead ends. These resistance patterns have shown large inter-regional variability.

Understanding the spectrum and resisitance patterns may help guide effective empirical antibiotic therapies, decrease treatment failure and costs. [3]. In most countries, several structures such as conferences of consensus on the proper use of antibiotics and drug commissions in Teaching Hospitals draw on knowledge of the services microbial ecology to define a reasoned use of antibiotics. Empiric antibiotic therapy is usually based on epidemiological data which are updated and adapted geographically, highlighting the importance of local and regular monitoring of bacterial resistance.

Although bacterial resistance to antibiotics is specific to each region, but no country can protect itself from the import of resistant pathogens through travel and trade [4]. The aim of this study was to assess the diversity and state of resistance of uropathogenic bacteria to antibiotics. Data were collected for a 12-year long period (2000–2011) at the Central Laboratory of Treichville Teaching Hospital (Abidjan, Ivory Coast).

Subjects and methods

Samples

During the study period, 12,175 urine samples from outpatients and inpatients were collected in different health services and analyzed. Under sterile condition, the midstream urine sample was collected on the first morning urine or on urination performed four hours later. These samples were quickly recorded and processed according to the routine protocol of urinalysis.

Samples processing

Microscopic examination of urine (0.1 mL) was performed to enumerate sample, leukocytes, erythrocytes, urothelial cells and crystals. The results helped to make the initial diagnosis of a possible urinary tract infection. Samples (5 mL) exhibiting more than 10^4 leukocytes per mL were centrifuged (5000 $\times g$ for 20 min at 4 °C) and pellets were stained with coomassie blue. These results were used to orientate the choice of isolation medium.

Urine sample (0.01 mL) was inoculated on CLED agar (Cystine-Lactose-Electrolyte-Deficient), which allows the growth of most uropathogenic bacteria. More selective isolation media were also used according to the results of the qualitative direct test. *Enterobacteriaceae* selection was made on Drigalski agar, MacConkey agar or EMB (Eosin Methylene Blue) agar. The Gram-positive cocci were selected on blood agar supplemented with colistin. The isolated bacteria were then identified by their biochemical characteristics using API galleries.

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined using the disc diffusion method on Mueller Hinton agar according to the guidelines of the Antibiogram Committee of the "Société Française de Microbiologie" (CA-SFM) [5].

Different families of antibiotics (discs obtained from Diagnostic Pasteur) were studied such as: cephems (cefuroxime, cefotaxime, ceftazidime, ceftriaxone); penicillins (amoxicillin, piperacillin; beta-lactam + inhibitor: amoxicillin/clavulanic acid); monobactam (aztreonam); quinolones (norfloxacin, ofloxacin, pefloxacin, nalidixic acid); aminoglycosids (gentamicin, netilmicin, tobramycin); tetracyclins (minocyclin, tetracyclin); trimethoprim/sulfamethoxazole; fosfomycin; lipopeptide (polymyxin B); chloramphenicol. After incubation, the diameter of the inhibition zone formed around the disc was measured and compared to the critical values "*d*" and "*D*" of each antibiotic disc to qualify the target bacteria as sensitive (diameter of the inhibition > *D*) or resistant (diameter of the inhibition < *d*) or again intermediate (*d* < diameter of the inhibition < *D*). Control tests were performed with reference

Table 1 Profile prevalence of uropathogenic bacteria isolated.

Bacteria isolated	Total number	(%)	From inpatients	(%)	From outpatients	(%)
Acinetobacter sp.	176	(5.7%)	80	(6.5%)	96	(5.3%)
Enterobacter aerogenes	307	(10.0%)	136	(11.0%)	171	(9.3%)
Enterobacter cloacae	124	(4.1%)	47	(3.8%)	77	(4.2%)
Enterobacter sp.	83	(2.7%)	37	(3.0%)	46	(2.5%)
Escherichia coli	879	(28.7%)	345	(28.0%)	534	(29.2%)
Klebsiella oxytoca	248	(8.1%)	87	(7.1%)	161	(8.8%)
Klebsiella pneumoniae	456	(14.9%)	221	(17.9%)	235	(12.8%)
Proteus mirabilis	80	(2.6%)	50	(4.1%)	30	(1.6%)
Pseudomonas aeruginosa	163	(5.3%)	75	(6.1%)	88	(4.8%)
Salmonella sp.	12	(0.4%)	6	(0.5%)	6	(0.3%)
Salmonella Typhi	2	(0.1%)	0	(0.0%)	2	(0.1%)
Staphylococcus aureus	534	(17.4%)	150	(12.2%)	384	(21.0%)
Total	3064	(100%)	1234	(100%)	1830	(100%)

strains *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

Data processing and statistical analysis

Data were analyzed with WHONET 5.6 software provided by the World Health Organization [2,6] to characterize and quantify the epidemiology of urinary tract infections. The percentage of antibiotic resistance, their evolution over time and the rates of multi resistant strains isolated were also assessed. Multi Resistant strains were divided into MDR (Multiple Drug-Resistant), XDR (Extensively Drug-Resistant) and PDR (Pandrug-resistant) according to the European Center for Disease prevention and Control [7]. MDR bacteria are defined as resistant to at least three different classes of antibiotics. XDR bacteria are characterized by their sensitivity to only one class of antibiotics and the PDR bacteria are resistant to all classes of antibiotics recommended for treatment.

Comparison between the rate of antibiotic resistance in strains from inpatients and outpatients was performed by chi-square (ξ^2). The Cochran–Armitage test was used to study the trends of antibiotic resistance over time. These statistical tests were performed using XLSTAT software 2009 version and the threshold for statistical significance was $p < 0.05$.

Results

Epidemiology of urinary tract infections

Of 12,175 urine samples processed, 3058 samples exhibited evidence of infection. Bacterial pathogens isolated (3071) belong to 12 species. *E. coli* (28.7%) was the commonest pathogen isolated with *S. aureus* (17.4%), *Klebsiella pneumoniae* (14.9%) and *Enterobacter aerogenes* (10%) (Table 1). These genera represent 71% of isolated bacterial strains. A majority of them were isolated from outpatients (59.6%)

Antibiotic resistance

Comparison of antibiotic resistance rates was performed specifically for *E. coli* and for all strains combined (Table 2). Very high rates of resistance to amoxicillin, tetracycline and trimethoprim/sulfamethoxazole were observed (close to 80%). Other antibiotics maintained their relative activity, such as ceftazidime, tobramycin, cefotaxime, aztreonan, polymyxin and especially netilmicin (3%). These results were observed both for *E. coli* and for all strains combined whatever they came from in- or outpatients. However antibiotic effectiveness declines over time (Fig. 1). The bacterial resistance rates to amoxicillin and cefotaxime dramatically increase respectively of 25% and 54.2% from 2000 to 2010 to reach close to 100%. Although the average rate of chloramphenicol resistance over 12 years was high, a decrease of 32.6% was observed from 2002 to 2010. *E. coli* and all strains combined exhibit similar trends of antibiotic resistance.

Multi-resistant strains

Based on their phenotypes, multi-resistant strains were classified by WHONET 5.6 software as MDR, possible XDR or possible PDR (Table 3). Most bacterial species presented a multiple drug resistance higher than 20%, particularly for *P. aeruginosa* (36.2%) and *Enterobacter* sp. (35.5%). The chi-square test showed that the rates of MDR and XDR strains from inpatients were significantly higher than those of outpatients (Fig. 2).

Discussion

Diversity of uropathogens

We compared the diversity of uropathogens isolated at Treichville Teaching Hospital (Abidjan, Ivory Coast) from 2000 to 2011 to that reported in similar studies from worldwide regions. As expected, *E. coli* was shown to be the commonest infecting uropathogen. However, we reported a lower prevalence of *E. coli* infection (less than 50%) as compared to European studies (over 60%) [8]. *S. aureus* and *K. pneumoniae* accounted for a higher fraction of isolates in our study than reported in European studies (less than 1%). Such results are similar to those reported from studies in sub-Saharan Africa [9–15] (Fig. 3). This specificity of uropathogenic bacteria diversity may be due by climatic factors, life conditions and habits of local population.

Antimicrobial resistance

The antimicrobial resistance profile of isolates in our study revealed a generally higher resistance rate than reported in European studies. Penicillins studied exhibited a resistance rate higher

Table 2 Number and resistance rates for different antibiotics.

Antibiotic subclass	Antibiotic	All strains combined					*Escherichia coli*				
		Inpatients		Outpatients		ξ^2 test	Inpatients		Outpatients		ξ^2 test
		Nb	*%R*	Nb	*%R*	*p*-Value	Nb	*%R*	Nb	*%R*	*p*-Value
Penicillins	Amoxicillin	328	78.9	574	75.7	0.276	92	84.7	164	85.3	0.810
	Piperacillin	178	38.7	247	26.7	**0.009**	53	47.1	80	26.2	**0.013**
	Amox/Cl. acid	480	48.7	541	43.4	0.089	151	41.7	161	34.1	0.169
Cephalosporin II	Cefuroxime	715	37.9	918	29.1	**<0.001**	222	22.0	320	18.1	0.256
Cephalosporin	Cefotaxime	410	13.9	567	14.8	0.688	135	6.6	222	12.1	0.095
III	Ceftazidime	694	15.5	889	13.5	0.246	240	8.7	350	10.8	0.402
	Ceftriaxone	100	21.0	187	11.7	**0.037**	43	16.2	91	8.7	0.199
Monobactam	Aztreonam	186	12.9	191	14.1	0.726	65	7.6	62	8.0	0.938
Fluoroquinolones	Norfloxacin	156	28.2	164	26.2	0.690	45	24.4	37	27.0	0.790
	Ofloxacin	137	27.7	260	20.0	0.080	37	24.3	65	21.5	0.746
	Pefloxacin	165	25.4	251	16.3	**0.023**	44	36.3	58	15.5	**0.015**
Quinolones	Nalidixic acid	199	48.7	210	35.7	**0.008**	64	42.1	65	33.8	0.329
Aminoglycosides	Gentamicine	227	17.1	422	9.0	**0.002**	69	17.3	153	7.1	**0.021**
	Netilmicin	811	3.0	1121	4.1	0.203	248	1.2	327	2.1	0.398
	Tobramycin	99	15.1	117	10.2	0.278	28	17.86	24	12.5	0.593
Tetracyclines	Minocycline	188	56.3	280	47.8	0.070	59	52.5	78	50.0	0.768
	Tetracycline	82	73.1	85	76.4	0.623	26	73.1	24	75.0	0.095
Folate pathway inhibitors	Trim./Sulf.	314	81.8	395	77.9	0.203	93	84.9	114	78.9	0.268
Fosfomycin	Fosfomycin	75	10.6	166	18.0	0.144	24	4.1	48	6.2	0.716
Polymyxin	Polymyxin B	216	12.0	207	12.5	0.870	76	6.5	101	8.9	0.569
Phenicole	Chloramphenicol	276	55.8	488	44.6	**0.003**	85	49.4	150	41.3	0.231

Note: Nb: number; *%R*: resistance rate.
p values in bold are considered as significant.

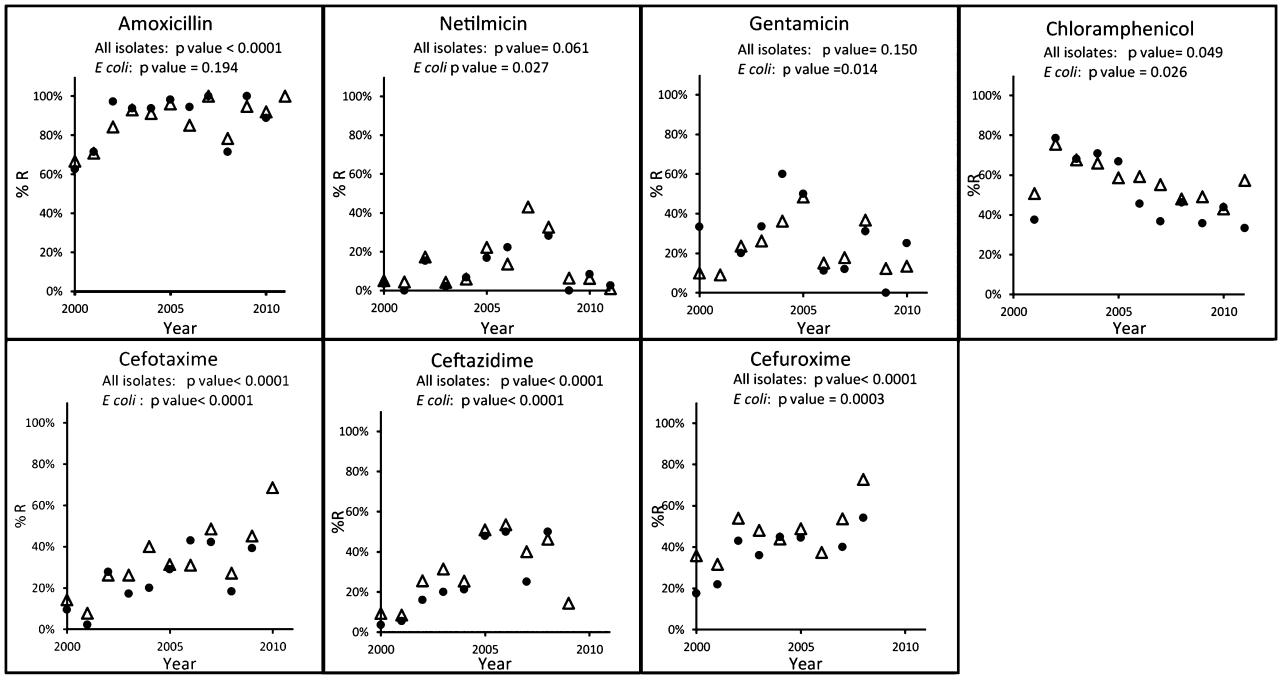

Figure 1 Trends in antibiotic resistance from 2000 to 2011 with Cochran–Armitage test.
Note: Filled circles: *Escherichia coli*, open triangles: all strains combined.

than cephalosporins, especially those of the third generation. We observed that resistance rates of *E. coli* from UTI to amoxicillin were the highest (85%). Such a strong resistance was observed worldwide [8,15–17]. This rate rose from 67% in 2000 to 91% in 2010. With *E. coli* strains, the rate of resistance increased from 62.5% in 2000 to 88.6% in 2010. Similar trend had also been observed in Dakar (Senegal) [17].

The combination of amoxicillin/clavulanic acid was more active than amoxicillin alone, but the observed resistance remained high

Table 3 Percentage of multidrug resistance for each identified species.

Isolates	Total number	MDR	(%)	XDR	(%)	PDR	(%)
Escherichia coli	879	127	(14.38%)	80	(9.06%)	11	(1.25%)
Staphylococcus aureus	534	32	(5.98%)	21	(3.93%)	8	(1.50%)
Klebsiella pneumoniae	456	106	(23.14%)	71	(15.50%)	14	(3.06%)
Enterobacter aerogenes	307	62	(20.20%)	45	(14.66%)	9	(2.93%)
Klebsiella oxytoca	248	61	(24.60%)	41	(16.53%)	11	(4.44%)
Acinetobacter sp.	176	59	(33.52%)	40	(22.73%)	15	(8.52%)
Pseudomonas aeruginosa	163	59	(36.20%)	48	(29.45%)	5	(3.07%)
Enterobacter cloacae	124	44	(35.48%)	33	(26.61%)	4	(3.23%)
Enterobacter sp.	83	30	(36.14%)	28	(33.73%)	0	(0.00%)
Proteus mirabilis	80	16	(20.00%)	10	(12.50%)	1	(1.25%)
Salmonella sp.	12	3	(25.00%)	3	(25.00%)	0	(0.00%)
Salmonella Typhi	2	0	(0.00%)	0	(0.00%)	0	(0.00%)
Total	3064	599	(19.51%)	420	(13.68%)	78	(2.54%)

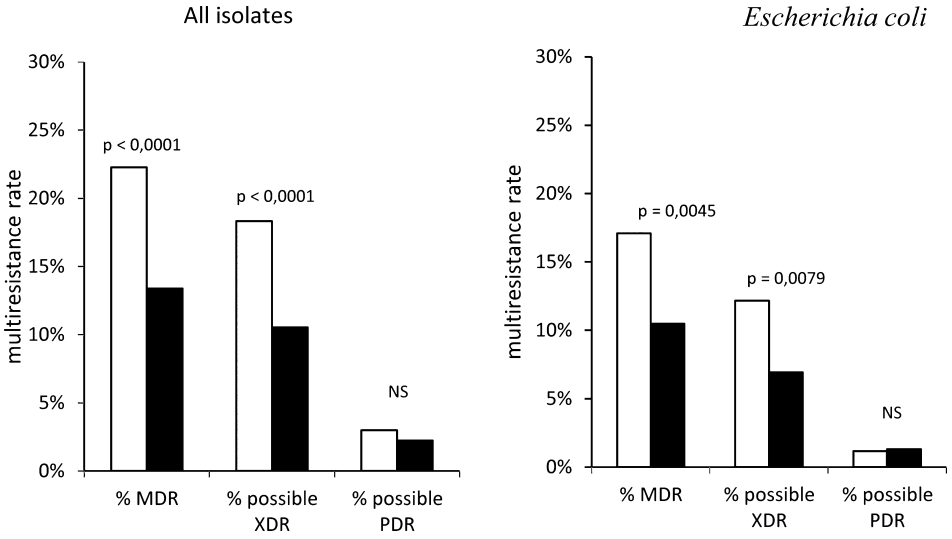

Figure 2 Comparing of the rates of multidrug resistant from outpatients versus those from inpatients with chi-square test.
Note: Filled rectangles: multiresistant from inpatients, empty rectangles: multiresistant from outpatients. NS: non significant.

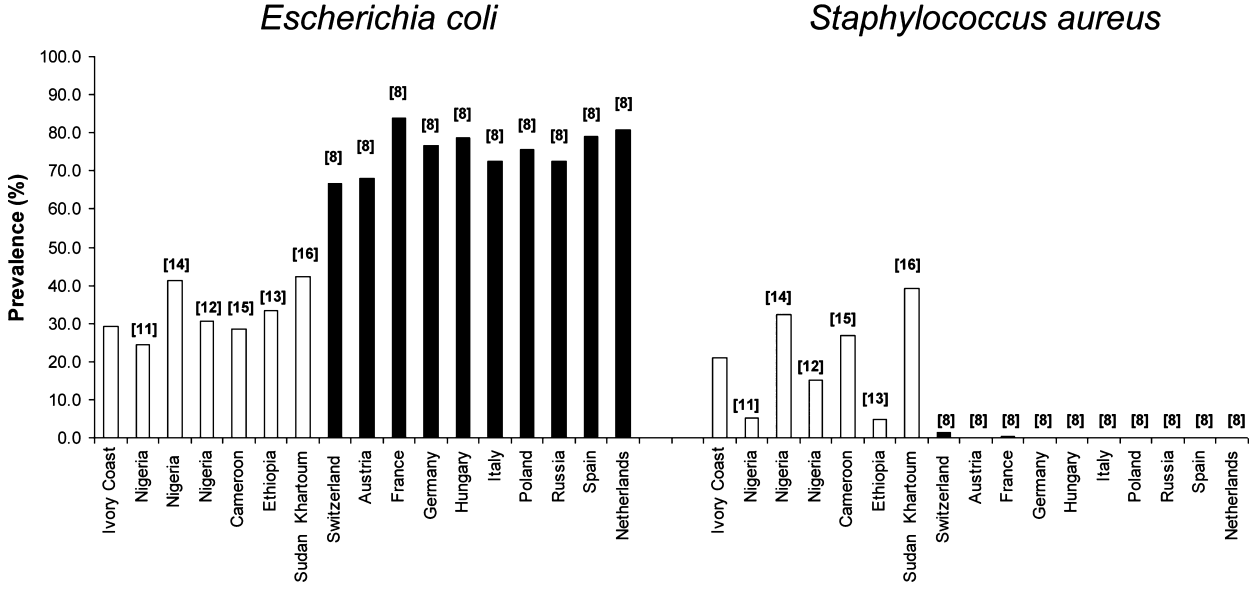

Figure 3 Regional specificity of prevalence of two uropathogenic bacteria (*E. coli* and *S. aureus*).
Note: Filled rectangles: European countries, empty rectangles: sub-Saharan African countries.

in Ivory Coast (34%). This value was lower than that observed in some African countries [15,17,18].

Third generation cephalosporins retained their relative efficiency. More than 80% of isolates were sensitive to ceftriaxone, ceftazidime and cefotaxime. Cephalosporin efficiency was lower than those observed in some countries in Africa and Europa [16,19]. These cephalosporins had retained some activity on uropathogenic bacteria in Ivory Coast and Africa, but the trend tests revealed that their efficiency was decreasing gradually over time. The rate of resistance to ceftazidime increased significantly from 3% in 2000 to 50% in 2008 (p-value < 0.0001). Resistance to cefotaxime evolved from 9% in 2000 to 39% in 2009 ($p < 0.0001$).

There was also a positive trend for the rate of resistance of *E. coli* to cefuroxime, a second-generation cephalosporin. This value evolved from 17% in 2000 to 54% in 2008 (p-value = 0.0004).

About the family of quinolones, the resistance rate of *E. coli* to nalidixic acid, to norfloxacin and to ofloxacin were less than those encountered in some African and European countries [10,20,21].

In the family of aminoglycosides, our results had shown a low rate of resistance of *E. coli* to gentamicin from outpatients (7%) as observed in Africa and in Europa [16,17,19,20]. We recorded a high rate of resistance to this antibiotic in some African countries such as Nigeria and Morocco [10,13,15]. The most recent one (netilmicin) appeared to be the most effective. The average rate of *E. coli* resistance during these 12 years was 2.14%. However the trend test performed had indicated a growth of the rate of resistance to this antibiotic (p-value = 0.02).

Chloramphenicol was used less often because of its toxicity. The average resistance to this antibiotic in Ivory Coast between 2000 and 2011 was 41% and has been decreasing over years. This resistance rate was lower than that observed in Nigeria in 2008–2009 (52%)[13].

Multi-drug resistant

In agreement with the consensus conference jointly organized by the French Infectious Diseases Society (SPILF) and the French Association of Urology (AFU), we had observed significantly higher percentage of MDR and possible XDR bacteria strains from inpatients than those of outpatients [22]. The uropathogens bacteria genus of *Acinetobacter* and *Enterobacter* were exhibiting the highest rates of MDR strain.

Conclusion

Our study had given an overview of the common uropathogens bacteria found in Ivory Coast and had shown a specific bacterial diversity in African countries compared to that of European countries. The spectrum of antibiotics activities showed that some antibiotics retain their efficiency on urinary tract infections such as third-generation cephalosporins, aminoglycosides, monobactams, lipopeptides and fosfomycin. The antimicrobial resistance increase overtime whatever their effectiveness. Moreover, high level of MDR and the emergence of XDR and PDR strains were observed.

In Ivory Coast as well as in other developing countries, some habits were already identified as actions which promote the emergence of resistant bacterial strains to antibiotics such as self-medication, supported by the sale of illegal drugs [23].

Conflict of interest

None to declare.

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