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Evolution Profile of *Escherichia coli* Resistance from January 2009 – April 2013 to Antibiotics at the Yaounde University Teaching Hospital, Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EELM and HKG designed the study, performed the statistical analysis and wrote the protocol. Authors EELM, EML, CGMK, WAB and HKG wrote the first draft of the manuscript and managed literature searches. Authors EELM, WAB and ABC managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Many strains of *Escherichia coli* (*E. coli*) have been proven to be pathogenic and are sometimes responsible for deadly outbreaks. This bacterium has become more resistant to antibiotics to which it is often sensitive. The aim of this study was to study the evolution of *E. coli* resistance to antibiotics from 2009 to 2013 at the Yaoundé University Teaching Hospital.
We included archived bench files containing information on patient's demographic data and results of antimicrobial susceptibility testing. The data were analyzed using Microsoft office, Excel 2007 software and SPSS.

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A total of 350 strains of *E. coli* were collected from both hospitalized and non-hospitalized patients. The antimicrobial susceptibility was tested using 23 antibiotics from January 2009 to April 2013 at the Bacteriology Unit of the Yaounde University Teaching Hospital. We observed a decrease in the trend of the resistance to 8 of the antibiotics tested: Amoxicillin + clavulanic acid, cefuroxime, cefoxitin, imipenem, ofloxacine, colistin, gentamicin and netilmicin. Meanwhile, we noticed an increase in the trend of resistance to 15 antibiotics: Amoxicillin, cephalothin, cefoxitin, cefotaxime, ceftazidime, cefixime, cefepime, aztreonam, amikacin, nalidixic acid, norfloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, nitrofurantoin, chloramphenicol, fosfomycin). The trend observed were statistically significant, for the resistance rate to amoxicillin + clavulanic acid (P value=0.002), also to resistance rates of amikacin and cefotaxime (P-values=0.008 and 0.014 respectively).
This increase in resistance over the years to most of the commonly used antibiotics has caused *E. coli* to be classified among multidrug resistant bacteria. In order to avoid a therapeutic impasse, it is necessary to carry out sensitization against the abusive use of antibiotics; surveillance activities for multidrug resistant bacteria and nosocomial infections should be reinforced as *E. coli* is one of a most common nosocomial bacteria.

Keywords: Escherichia coli; multidrug-resistance; evolution; antimicrobial resistance.

1. INTRODUCTION

E. coli is the predominant germ of commensal aero-anaerobic flora of the gastrointestinal tract of humans and animals [1]. However, some strains can be pathogenic. Many studies have shown the morbidity and mortality of pathogenic strains of *E. coli* in several regions of the world. In Toukam et al. [2] research, *E. coli* was the most prevalent Enterobacteriacea isolated from clinical specimens from hospitalised and community patients at the Yaoundé General Hospital.

According to WHO, enterotoxinogenic *E. coli* (ETEC) causes 210 million cases of diarrhea every year with severe epidemics and 380000 deaths in developing countries [3]. Its role in urinary tract infection is widely documented; in fact *E. coli* is incriminated in almost 60 to 80% of this infection [4,5].

E. coli like other bacteria show resistance to antibacterial through intrinsic or acquired resistance mechanism [6]. The intrinsic resistance refers to existence of resistance genes as part of the genome encoding mechanisms intrinsically found in the population of the bacteria. In contrast, the acquired resistance mechanisms are attained by bacteria through mutations or mechanisms of horizontal gene transfer such as conjugation, transformation, and transduction. Generally bacteria show evidence of biochemical resistance by three different mechanisms: by reducing their permeability into the cell and/or by active efflux mechanism; by structurally altering the antibiotic targets by enzymatic or non-enzymatic modification or inactivation of the antibiotic before reaching the targets. Enzymatic mechanism is one of the most common resistance mechanism [6]. Among enzymes, there are extended spectrum β-lactamases (ESBLs), enzymes belonging to 2be of Bush, Jacoby, and Medeiros Subgroup. They are active against β-lactamases except cephamycin and carbapenem. ESBLs become more and more incriminated as one of the common and frequent mechanisms of *E. coli* [7,8].

The evolution of bacterial resistance henceforth constitutes a major risk of public health, because, amongst the several pathogenic species, certain strains are sensitive to only a few antibiotics. The consequences are numerous: increased morbidity and mortality, increase in health care costs related to prolonged hospitalization and hence the need for more costly and often more toxic antibiotics. Some strains are resistant to all the antibiotics usually available on the market. The control of the appearance and the extended resistance of pathogens to antibiotics has become imperative for medical laboratories so as to establish a useful data base and initiate an epidemiologic surveillance for resistance. Tunisia and Morocco, have noticed an increase in the resistance of *E. coli* to antibiotics [6,7]. In Cameroon, studies carried on antimicrobial resistance of isolates of gram negative bacilli in hospitalized and ambulatory patients in the Yaounde Central Hospital (YCH) from April 1995 to March 1998 showed that *E. coli* and *Klebsiella* species were resistant to cephalosporines of the third generation and other beta lactamines by the production of high and low level penicillinase and extended spectrum β-lactamase [8]. Likewise, in

the bacteriology laboratory of the Yaounde University Teaching Hospital (YUTH) high frequencies of resistance to antibiotics were also found with: amoxicillin, cefalotine and cotrimoxazole in 2008 [9]. Considering the fact that bacterial resistance is a dynamic phenomenon, susceptible to evolve with time and space, the objective of our study was to provide information about the evolution of *E. coli* resistance to antibiotics in YUTH since 2009.

2. MATERIALS AND METHODS

This was a cross sectional descriptive retrospective study from January 2009 to February 2013 and prospective from March to April 2013. The data was collected at the bacteriology laboratory of YUTH. The sampling method was convenience sampling. All strains of *E. coli* were isolated and identified from hospitalized and non-hospitalized patients, using standard biochemical techniques. The antimicrobial susceptibility testing of the *E. coli* strains were determined using the disk diffusion method (Kirby Bauer) in Mueller Hinton agar medium, and the choice of antibiotic used was according to the Committee of the French society of microbiology (CA-SFM). Quality control of culture media and antibiotic disks were carried out using the ATCC 25922 *E. coli* reference strain.

3. RESULTS

A total of 350 strains of *E. coli* were collected. Among the pathological samples, urines, pus and blood were the most prevalent as shown on Fig. 1. Among the hospitalized patients, Pediatrics and Intensive care unit were the most prevalent as shown on Fig. 2.

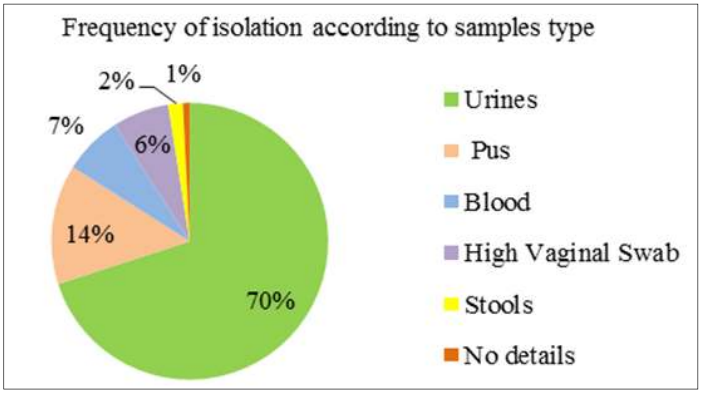

Fig. 1. Frequency of isolates according to samples type

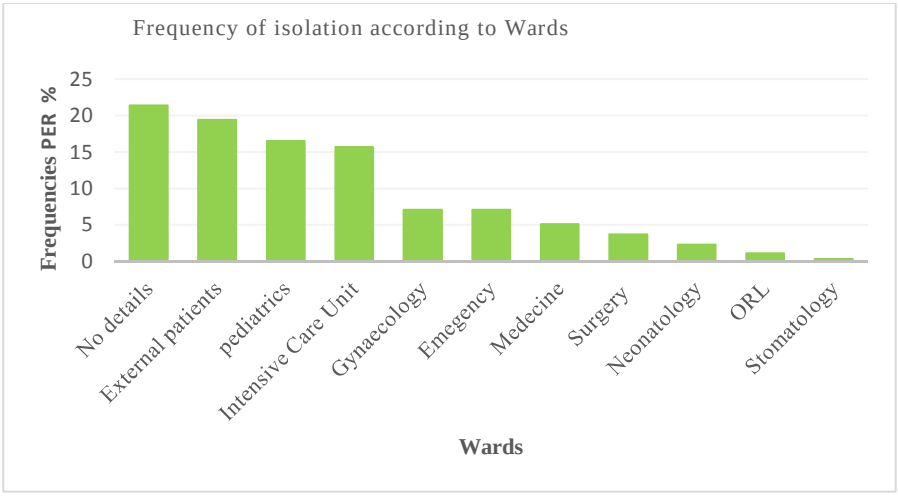

Fig. 2. Frequency of isolation according to wards

Figs. 3 to 7 present the trend curves of evolution of *E. coli* resistance against each family of antibiotics.

Among the β-lactamins, the trend of the resistance evolution curves for amoxicillin, cephalothin, cephotaxime, ceftazidime, cefixime and cefepime show an increase (shown on Fig. 3)While evolution curves for amoxicillin + clavulanic acid, cefuroxime, cefoxitin and imipenem show a decrease as presented in Fig. 4.

Among aminoglycosides, the trend of the resistance evolution curves for gentamycin and netilmicin showed a decrease while that of the amikacin showed an increase.

Considering evolution of resistance of *Escherichia coli* to the quinolones the resistance curve of ofloxacin shows a decrease whereas that of nalidixic acid, norfloxacin and ciprofloxaxin show an increase (Fig. 6).

Among the last group classed as "other antibiotics family" only the resistance curve of colistin show a decrease while the trend of aztreonam, trimethoprim-sulfamethoxazole, nitrofurantoin, chloramphenicol, fosfomycin curves present an increase. As presented in Fig. 7.

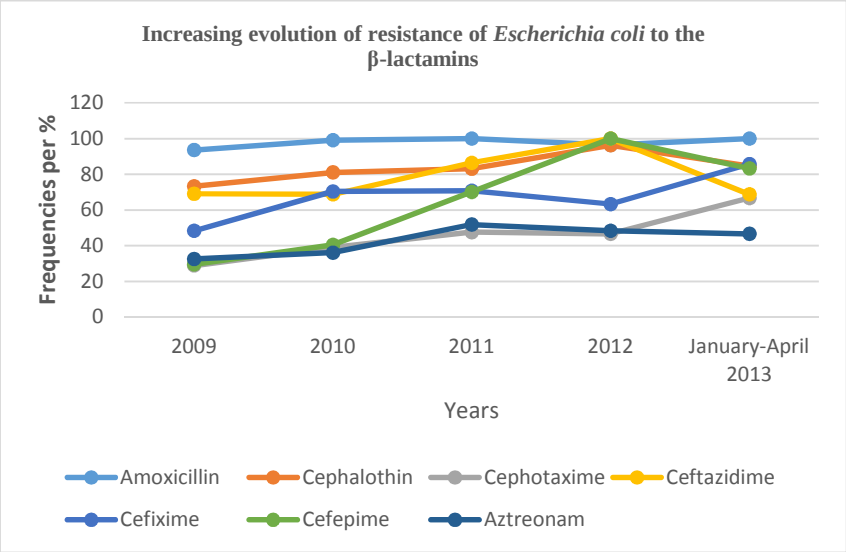

Fig. 3. Evolution of resistance of *Escherichia coli* to β-lactams

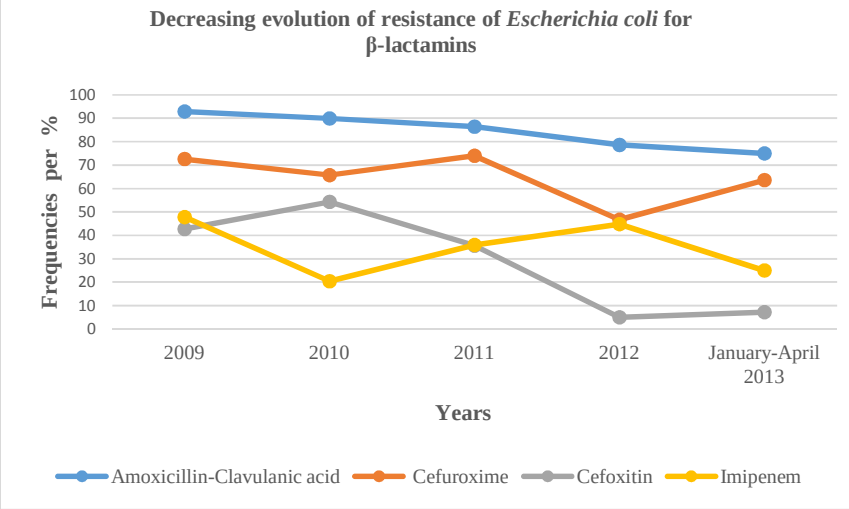

Fig. 4. Evolution of resistance of *Escherichia coli* to β-lactams

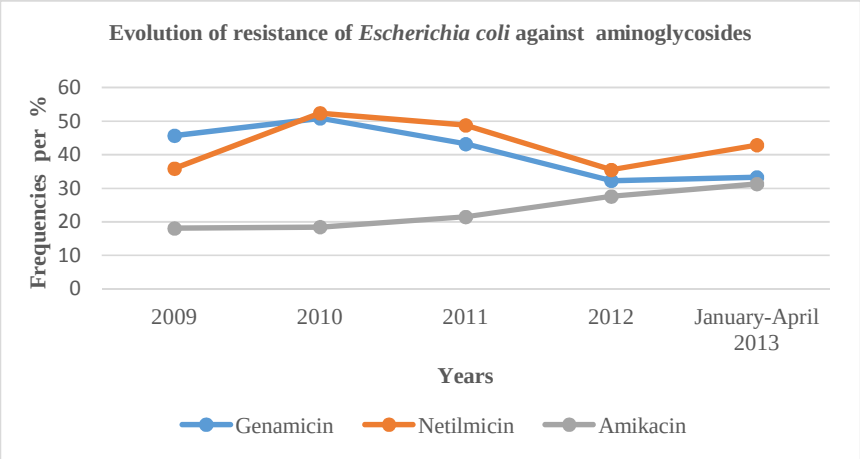

Fig. 5. Evolution of resistance of *Escherichia coli* to the aminoglycosides

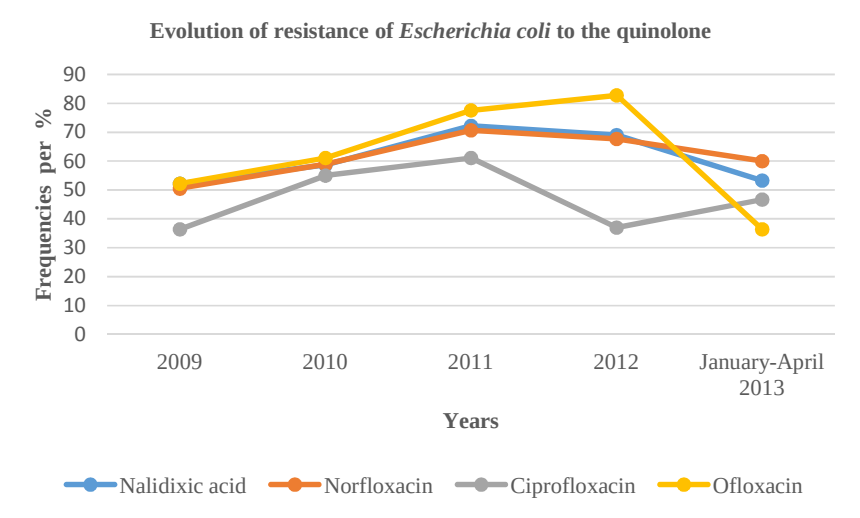

Fig. 6. Evolution of resistance of *Escherichia coli* to the quinolones

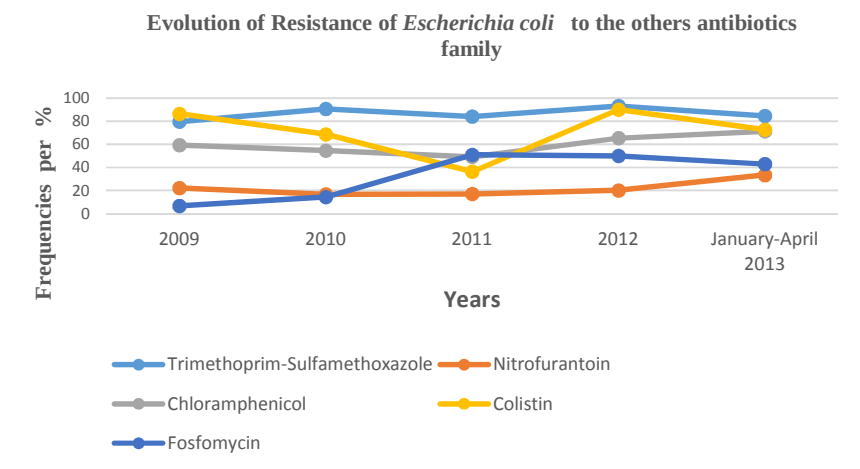

Fig. 7. Evolution of *Escherichia coli* resistance to other antibiotics family

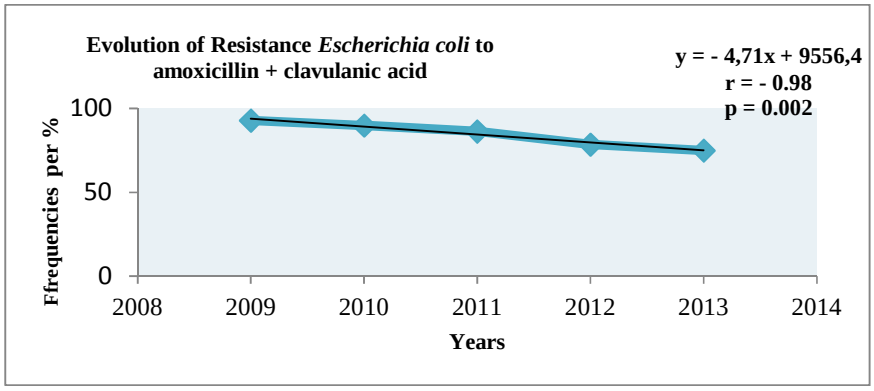

Fig. 8. Evolution of *Escherichia coli* resistance to amoxicillin + clavulanic acid

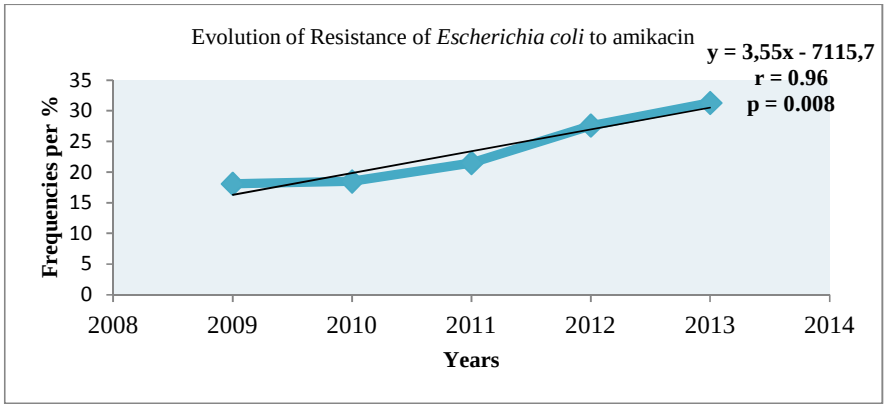

Fig. 9. Evolution of resistance of *Escherichia coli* to amikacin

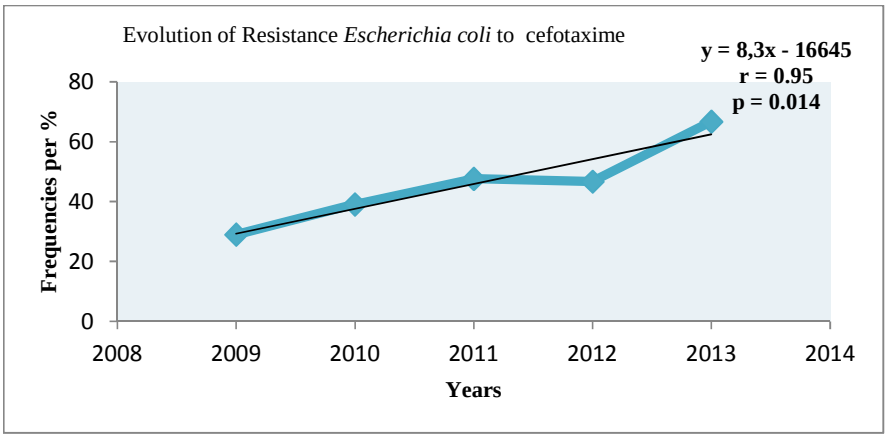

Fig. 10. Evolution of resistance of *Escherichia coli* to cefotaxime

From 2009 to 2013, the decrease in the resistance rate of amoxicilin + clavulanic acid, was statistically significant (P-value=0.002) while the increase in the resistance rate of amikacin and cefotaxime was also statistically significant with P-values=0.008 and 0.014 respectively as shown on figures 7, 8 and 9 respectively.

4. DISCUSSION

Many studies have shown that *E. coli* is the bacteria that is mostly incriminated in urinary tract infection: 66.4% in 2006 in Mali [4]; 76% in 2008 in Tunisia [9]. In our study, *E. coli* was also most frequently (70%) isolated in urine. This was

followed by pus 49 (14%) and blood 24.15 (6.9%); this is similar to the results obtained by Gangoué et al. [10] who revealed 65% in urine, 33.7% in pus and 1.3% in blood.

No strain was isolated from cerebriospinal fluid. These results are similar to that obtained by Fonkoua et al. [11] at *Centre Pasteur du Cameroun (CPC)*, on bacterial meningitis from 1999 to 2000, which showed that, out of 1872 cerebriospinal fluid analysed, only 1 strain of *E. coli* had been isolated from a man 81 years of age.

Concerning β-lactams, especially the penam sub-group, the resistance of *E. coli* to amoxicillin showed an increase throughout the period of study, reaching 100% in 2011 and 2013. The annual average of resistance was 97.5%. These results are similar to those of Njehawobe et al. (94%) [12] and Zomahoun 91.7% [13]. Resistance to amoxicillin+clavulanilic acid showed a reduction from 2009 but the annual average resistance remained high 88.4% compared to previous studies carried out by Kenkouo (76.6%) [14], Seck (61.1%) [15], Gangoue et al. (57%) [16] and Bourjilat et al. (57.6%) [17]. For the cephem sub-group; resistance to cephalothin a first generation cephalosporine, showed an increase throughout the 5-year study period. It rose from 73% in 2009 to 84.6% in 2013 with an important annual average resistance of 80.9%. This frequency was higher than the one obtained in two Cameroonian studies: [12] (73%) and [14] (10.9%). For the resistance to second generation cephalosporins like cefoxitin, there was a decrease in the resistance throughout the study years. Nevertheless, there was an annual average resistance of 31.16% which was higher than what was obtained in previous studies: 22% of resistance in Morocco [17]; 14.9% at CPC [14]; 9.6% in Cotonou [13]; 9% at YCH [16]; 8% at YUTH [12]; 5.75% in Mali [4] and 3.7% in Tunisia [9]. The resistance to cefuroxime decreased throughout the 5-year study period. The average annual rate of resistance was 67.9%. Nevertheless we observed a marked increase from 29% in 2008 [14] to 63.6% in 2013.

Concerning the third generation cephalosporins, resistance of *E. coli* isolated at YUTH from 2009 to 2013 to ceftazidime was higher than that obtained in previous studies with an annual average of 75.4%. Thus, 17% of resistance was found in 2008 [9], 10.9% of resistance CPC [14], 8% of resistance in HCY [17] and 5% of resistance in Morocco [17]. Resistance to cefixime also showed an increase from 2009. This resistance reached its highest value (85.7%) in 2013 and its average value for the study period was 63.9%. This value was higher than that found in other studies: 17% of resistance at YUTH [12] and 14.4% of resistance at CPC [14]. Resistance to cefotaxime showed a significant increase with an annual average of 67.9%. It rose from 29% in 2008 [12] to 63.6% in 2013.

For cefepime a fourth generation cephalosporin, the resistance of *E. coli* showed an increase with an annual resistance of 53.8%.This value was higher than that obtained in Mali in 2006 (3.45%) [4].

In general, we found that the resistance of *E. coli* to cephalosporins increased in YUTH.

The resistance to imipenem from 2009 to 2013 showed a decrease with an average of 34.4% thoughout the study period. However, this resistance to imipenem in YUTH is 30 times greater than that of other studies: 0% resistance in Tunisia [9] and YUTH [12]; 1% resistance in YCH [16].

The susceptibility of three antibiotics of the aminoglycoside family was tested: gentamicin, netilmicin and amikacin. The average frequency of resistance to amikacin was the lowest. The resistance to gentamicin and netilmicin showed a decrease. The resistance to gentamycin shows a decrease from 45.7% in 2009 to 33.3% in 2013 with an annual resistance of 45%. In spite of this decrease, our average annual resistance compared to others studies was still high: 25% resistance at YUTH [12], 19.5% resistance at Cotonou [13], 16.69% resistance in Mali [4]; 10% resistance in Morocco [10]; 5.3% resistance in Tunisia [9]. For netilmicin the annual average resistance was 44.8% and was higher than that obtained in Benin 15% [14] and at YUTH (7%) in 2008 [12]. The resistance to amikacin showed a significant increase from 2009. The annual average resistance of 20.6% was close to that found in some African studies: 14% resistance at YUTH [12]; 12.64% resistance in Mali [4]; 6.4% resistance at CPC [14] and 1.3% resistance in Tunisia [9].

In general, we notice that resistance to quinolone was higher (60-63%) than that of aminoglycoside (20-45%) but less than that of β-lactamine (40-97%). The resistance to nalidixic acid increased

every year. The average resistance was 60.6%; and was higher than Malian study 37.93% [4]. The resistance to norfloxacin increased from 2009 with an average resistance of 60.1%. Compared to those of some others studies we notice that in spite of the increased resistance observed, it remained lower than the 77.9% of Zomahoun's study [13] and was higher than the 37.93% of Ya Bi Foua's study [4]. The same trend was observed for norfloxacin. The resistance to ciprofloxacin in spite of the increase, its annual average (46.7%) was lower than the other study 78.2% [13]; but it remained also higher than some like Ya Bi Foua study 36.78% [4]. Among the quinolones, the resistance to ofloxacin showed a decrease with an annual average resistance of 36.4%. This average was higher than those observed by Kenkouo (23.9%) [14] and Gangoué-Pieboji et al. [16] (9%). For the sulfamides, resistance to trimethoprim- sulfamethoxazole increased constantly from 79.7 % in 2009 to 84.6% in 2013 with a peak of 93% in 2012 and 90% in 2010. The average resistance was 88.6%. These results are similar to those of [14], [16], [4], and [15] with 88%, 85.4%, 80.46% and 77.9% respectively.

For the fosfomycin family the resistance of fosfomycin showed an increase from 6.6% in 2009 to 42.9% in 2013. The average resistance was 30.7%. This result remains high compare to those of [4] 2.3% and those of [16] 2.6%.

5. CONCLUSION

Most of *E. coli* strains were more found in urine, pus and blood. The higher frequencies of samples came from in patients especially from the Intensive Care Unit, Emergency, Medicine, Surgery and Neonatology wards. Evolution of resistance to most antibiotics has increased since 2009, mostly to betalactams.

Given the scope of this dangerous dilemma facing the medical world which is the increase of bacteria resistance, urgent action is required. As far as the risk of treatment failures multiply exponentially and given that resistance to antibiotics and the various factors that have enabled that resistance to develop requires a multidisciplinary and multisectoral approach, it is up to each actor involved to assume its share of responsibility. This is intended to inform the public on the risks of uncontrolled use of antibiotics and to set up, particularly, on nosocomial bacteria a surveillance program knowing that *E. coli* was more isolated in hospitalized patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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