Antimicrobial Susceptibility of Community Acquired 
*Escherichia coli* in Urinary Tract Infections (UTI) in Benin for Eleven Years (2005-2015)

**Abstract:** Antimicrobial resistance became a growing public health problem in the world and *Escherichia coli* (*E. coli*) appeared as one of nine bacteria commonly causing infections in community and hospitals. It prevalence and its resistance to antibiotics were evaluated in Benin throughout an eleven-year period. In this retrospective study, routine urine samples from patients collected at the National Laboratory (NL) of Health Ministry during the period 2005-2015 were analyzed. Samples higher than 10³ CFU/mL bacterial growth were considered positive and for these cases, the bacteria were identified and Antimicrobial Susceptibility Test (AST) was performed. From the 4467 samples analyzed, 1455 (32.6%) were positive with *E. coli* preponderance (38.3%) of all isolated germs and (58.5%) of enterobacteriaceae. Most of the isolates were susceptibility to netilmicin (80%), gentamycin (93%), chloramphenicol (70%), pipemidic acid (60%), nalidixic acid (75%), ciprofloxacin (75%), amoxicillin/clavulanic acid (80%) and nitrofurantoin (100%). Susceptibility rates increased for cefotaxim (78-100%), ceftriaxon (71-100%) and aztreonam (67-100%). Resistances were observed for minocyclin (70%), Trimethoprim/Sulfamethoxazole (TMP-SMX) (60%), ampicillin (67%), amoxicillin (75%), carbenicillin (86%), cephalothin and cephalaxin (50 and 80% respectively). The use of drugs such as minocyclin, ampicillin, amoxicillin, carbenicillin, cephalothin and trimethoprim/sulfamethoxazole does not seem appropriate for empirical treatment of UTI in Benin.

**Keywords:** Community, Urinary Tract Infections, *Escherichia coli*, Antimicrobial Effectiveness, Benin

**Introduction**

Antimicrobial resistance had become a growing public health problem in the world and *E. coli* appeared as one of nine bacteria commonly causing infections in community and hospitals (WHO, 2014). Worldwide, *E. coli* remains the most encountered pathogen among those implicated in community acquired urinary tract infection (Jean-Marie *et al.,* 2007). With (46.4-74.2%) of global representation (Inês *et al.,* 2013), it’s the first
strains isolated in children between 2 to 5 years of age followed by Klebsiella, Proteus and Pseudomonas (Rajiv et al., 2013). In a recent study done in 2012 at Ngaoundere in Cameroon focused on community acquired infection, Carine et al. (2012) found that 66.7% of *E. coli* was mostly Extended-spectrum beta-lactamase producers in the samples of stools. It is well known the proximity between anal cavity and urinary tract in women. Urinary Tract Infections (UTI) is the most commonly diagnosed infections in communities which are often treated with different antibiotics. Some studies have reported an increasing resistance rate to antibiotics such as amoxicillin, amino penicillin, trimetoprim-sulfamethoxazole and others reducing therapeutic possibilities (Gupta et al., 1999; Kahlmeter, 2003a).

A study conducted in Benin have shown, that the beta lactam antibiotics were the most prescribed and represented 44.4% of prescriptions, followed, in order of imidazole, quinolones, aminoglycosides, sulfonamides, macrolides and related, cyclins and phenicol (Dissou et al., 2009). However, in Benin, few documentations exist in antimicrobial resistance of uropathogen isolated in UTI. The aim of this study was to describe the bacterial profile of UTI and antimicrobial susceptibility of *E. coli* strains isolated from the urine cultures of Benin patients in National Laboratory (NL) of Heath Ministry over 11 year period.

### Methods

Routine urine samples data were collected from patients in Cotonou between 1st January 2005 and 31st December 2015 period. This study was approved by the Research Ethics Committee for Applied Biomedical Sciences (CER-ISBA) of Abomey Calavi University of Benin. A retrospective cross sectional study was conducted in NL where urine samples were analyzed. For each patient we extracted from the laboratory records, year, age, sex, early urine culture results, identification of the bacterial strain responsible of UTI and the corresponding Antimicrobial Susceptibility Test (AST) results.

The statistical test EpiData 3.1 was used to check-in results and EpiData Analysis V2.2.2.182 for data analysis. Data were expressed an absolute values and percentage. The chi-2 test was used to compare proportions. To be significant, the p value should be less than 0.05.

### Results

Overall 4467 urine cultures were performed during 2005-2015 period. The rate of positive cultures was 32.6% (1455/4467) of which 1470 bacteria have been isolated. Characteristics of patients included in the study are shown in Table 1. Mean age and sex ratio (M/F) of study population were 30.5 years [95% Confidence Interval (CI) 30.01-30.98] and 0.5. Frequencies and distribution of isolated uropathogenic bacteria during the eleven study years were summarized in Table 2. The enterobacteriaceae proportion was 65.5% (963/1470) with *E. coli* prevalence which represented 38.3% (563/1470) of all isolated bacteria and 58.5% (563/963) of enterobacteriaceae. The others most represented isolated bacteria were Klebsiella pneumoniae 23.4% (344/1470), Staphylococcus aureus 16.0% (235/1470), Staphylococcus spp 7.6% (111/1470), Staphylococcus dore 3.7% (54/1470).

The Fig. 1 shows the susceptibility rates of *E. coli* for aminoglycosides, tetracycline, phenicol, quinolones, sulfonamides+associations, penicillin, cephalosporin, aztreonam and nitrofurantoin.

Regarding aminoglycosides, we observed susceptibility increase of *E. coli* to netilmicin (10 µg) (80 to 100%) on three use years. During the 11 study years, the maximum susceptibility rate to gentamycin (30 µg) was 93.3%.

Since 2007, *E. coli* susceptibility to tetracyclin (30 µg) decrease to 12% at 2015. Concerning minocycin (30 µg), on three use years, his susceptibility rate was 30%.

Among phenicol, chloramphenicol (30 µg) was one used during the first ten years, *E. coli* susceptibility to this drug varied between (38 to 70%) until 2014. Among quinolones, pipemidic acid (20 ug) was tested during three years (2005-2007) with *E. coli* susceptibility rate not exceeded 60%. The others same class antibiotics used (2005-2015) were nalidixic acid (30 µg) while the maximum *E. coli* susceptibility rate was 75% and ciprofloxacin (10 ug) while susceptibility rate increased and reached 100% at 2010 and decreased to 75% at 2015.

For sulfonamides+associations (25 ug) class, *E. coli* susceptibility to Trimethoprim/Sulfamethoxazole (TMP-SMX) not exceeded 40% (2005-2011). This susceptibility decrease from 2011 to 2015 (at 0%). Four penicillin class of antibiotics have been tested. Ampicillin (30 ug) has been used during two years (2005 and 2014) and *E. coli* susceptibility rate around 33%. During the 11 study years and the same class, amoxicillin (25 ug) was less active to *E. coli* while susceptibility rate not exceeded 25%. As for Amoxicillin-Clavulanic acid (AMC) (30 ug), the most tested antibiotic in this class, effectiveness was observed until 2009 (80%). But from this year, a loss effectiveness was observed until 2015 (62%). On three use years, carbenicillin (100 ug) was less active to *E. coli* while susceptibility rate not exceeded 14%.

| Characteristic | All patients (N = 4467) |
|---------------|------------------------|
| **Sex**       |                        |
| Male          | 1576 (35.28%)          |
| Female        | 2891 (64.72%)          |
| **Age (years)** |                       |
| Minimum       | 1                      |
| Maximum       | 95                     |
| Mean          | 30.50                  |
| **Cultures**  |                        |
| Positive      | 1455 (32.57%)          |
| Negative      | 3012 (67.43%)          |
Table 2. Percentage and distribution of isolated uropathogenic germs during the eleven study years

| Bacteria                        | n (%)   | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---------------------------------|---------|------|------|------|------|------|------|------|------|------|------|------|
| Escherichia coli                | 563 (38.3) | 119 | 118 | 67  | 28  | 34  | 35  | 16  | 45  | 53  | 40  | 8   |
| Klebsiella pneumoniae           | 344 (23.4) | 49  | 40  | 29  | 29  | 32  | 25  | 25  | 48  | 33  | 22  | 12  |
| Staphylococcus aureus           | 235 (16.0) | 38  | 47  | 28  | 10  | 26  | 10  | 13  | 22  | 21  | 17  | 3   |
| Staphylococcus spp              | 136 (9.7)  | 0   | 8   | 7   | 10  | 7   | 11  | 24  | 23  | 29  | 23  | 9   |
| Staphylococcus dore             | 54 (3.7)   | 50  | 2   | 1   | 0   | 0   | 0   | 1   | 0   | 0   | 0   | 0   |
| Streptococcus β hemolytique     | 36 (2.4)   | 2   | 5   | 4   | 5   | 2   | 4   | 2   | 3   | 2   | 7   | 0   |
| Pseudomonas spp                 | 23 (1.2)   | 8   | 5   | 2   | 1   | 1   | 2   | 0   | 2   | 1   | 1   | 0   |
| Staphylococcus citrin           | 13 (0.9)   | 12  | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Pseudomonas aeruginosa          | 13 (0.9)   | 0   | 3   | 3   | 0   | 1   | 2   | 0   | 0   | 1   | 2   | 1   |
| Klebsiella oxytoca              | 13 (0.9)   | 0   | 0   | 2   | 1   | 3   | 2   | 1   | 3   | 1   | 0   | 0   |
| Citrobacter spp                 | 10 (0.7)   | 1   | 3   | 2   | 0   | 0   | 1   | 0   | 2   | 1   | 0   | 0   |
| Proteus mirabilis               | 7 (0.5)    | 1   | 1   | 2   | 1   | 0   | 1   | 0   | 1   | 0   | 0   | 0   |
| Candida albicans                | 4 (0.3)    | 0   | 1   | 2   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Proteus vulgaris                | 4 (0.3)    | 0   | 2   | 0   | 1   | 0   | 0   | 0   | 0   | 1   | 0   | 0   |
| Levinea                         | 4 (0.3)    | 3   | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Proteus morganii                | 3 (0.2)    | 2   | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Bacille pyocyanique             | 2 (0.1)    | 2   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Edwardsiella                    | 1 (0.1)    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Arizona rhinocromatis           | 1 (0.1)    | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Listeria monocystogene          | 1 (0.1)    | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Proteus rettgeu                 | 1 (0.1)    | 0   | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Levinea malonatica              | 1 (0.1)    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Streptococcus faecalis          | 1 (0.1)    | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   |
| Total                           | 1470 (100.0) | 289 | 234 | 152 | 86  | 112 | 88  | 69  | 151 | 138 | 118 | 33  |
About cephalosporin, four antibiotics were tested: cephalothin (30 ug), cephalexin (30 ug), cefotaxim (30 ug), ceftriaxone (30 ug). Cephalothin and cephalexin have been tested three years each. *E. coli* susceptibility rate to these drugs was 50% and 20% respectively. Cefotaxim and ceftriaxone were very effectiveness and were mainly tested of antibiotics. Their effectiveness rate to *E. coli* reached 100% to 2010. From this year, these effectiveness have loss until 2015 with 33.3% and 37.5% rate respectively. *E. coli* developed resistance to cephalothin and cephalexin (50% and 80% respectively).

Aztreonam (30 ug) tested during six years showed 100% rate effectiveness to *E. coli*. Following a decrease in 2008, a continuous increase in susceptibility rates was observed until 2010 (100%).

Among nitrofurans tested, nitrofurantoin (300 ug) using till 2014, showed a good effectiveness with 100% rate.

**Discussion**

*E. coli* appeared as the most frequent bacteria in UTI representing 38.3% (563/1470) of all isolated bacteria and 58.5% (563/963) of enterobacteriaceae. The second major finding in our study was *E. coli* susceptibility to antibiotics.

In Benin, UTI were major causes of antibiotics prescribing among adults and adolescences (Dissou et al., 2009). The lack of national recommandations involve their bad utilization (Dissou et al., 2009). In fact, Benin is West Africa country where a lot of counterfeit drug are circulating. A Pharmaceutical desposit drug is in Cotonou at Dantokpa market. Thus, the population supply drug and taking care by self-medication. In addition, due to their results quality, the NL received widely patients for uroculture analyzes. This should explain the 32.6% rate positive uroculture observed in this study. This rate was two fold higher that observed in Senegal (Diop-Ndiaye et al., 2014) and Portugal (Inês et al., 2013). The major bacteria isolated in those studies was *E. coli* as in our study (38.3%) which the virulence factors are specific properties. Those specificity confer on the bacterium the ability to adhere to the urinary tract and invade the host tissues causing injury (Moura et al., 2009). This study shows a proportion of *E. coli* (38.3%, 563/1470), significantly lower than that observed by Nisel et al. (2016) (67%, 8975/13281).

Antibiotics resistance has increased in alarming proportions (WHO, 2001a; 2001b). Thus, their effectiveness for UTI treatment became quite limited (Temesgen et al., 2015). In our study, among amino glycosides, netilmicin showed susceptibility factors above 80%. This rate was similar those observed in Portugal and Bangui (98.7 and 96% respectively) (Hadiza et al., 2003; Inês et al., 2013). As for gentamycin, frontline antibiotic in the treatment of gram negative bacteria, its efficacy was 93% equal almost those observed in India, Senegal and Bangui (83.6, 93.8, 94% respectively) (Hadiza et al., 2003; Jean-Marie et al., 2007; Rajiv et al., 2013).

As for tetracycline, from 2007 year, the resistance rate around 76%. The loss effectiveness was very
alarming and this antibiotic should not be prescribed against UTI. At Pakistan, a similar resistance rate of *E. coli* to tetracyclin was observed in 70% (Ahmad *et al.*, 2015). Minocyclin in the first three years showed effectiveness around 30% while a study conducted in China reported 92.1% of susceptibility by (Haihong *et al.*, 2015).

The maximum efficacy rate of chloramphenicol to *E. coli* was 60%. Behailu *et al.* (2016) reported in Dil Chora Referral Hospital, Dire Dawa, Eastern Ethiopia an effectiveness rate of 77.8% in study conducted among pregnant women attending at antenatal clinic (Behailu *et al.*, 2016).

Over 11 year, nalidixic acid had maximum efficacy rate at 75%. This rate was lower than observed at Bangui (90%) (Hadiza *et al.*, 2003). However, none difference has been observed between the rate that of Dakar (76.1%) (Jean-Marie *et al.*, 2007). This antibiotic was the first among quinolone used only for the therapy of gram-negative UTI, because it has ability to penetrate the tissues (Moura *et al.*, 2009). Of high susceptibility until 2010, the loss effectiveness of ciprofloxacin should be explain by its large use.

Ciprofloxacin can be used in UTI patients with allergies to this antibiotic, that should be explained by its no medically needed. This antibiotic was the first among quinolone used only for the therapy of gram-negative UTI, because it has ability to penetrate the tissues (Moura *et al.*, 2009). Of high susceptibility until 2010, the loss effectiveness of ciprofloxacin should be explain by its large use. In fact, ciprofloxacin can be used in UTI patients with allergies to others drug or in old age patients with recurrent infections and in diabetics (Eom *et al.*, 2002; Schilling *et al.*, 2002; Killgore *et al.*, 2004). Nevertheless, the 75% susceptibility rate of *E. coli* observed in 2015 was not significant (p = 0.811) that observed in Dakar (Jean-Marie *et al.*, 2007).

Until 2011, forty percent of *E. coli* were susceptibility to TMP-SMX. This antibiotic was also called co-trimoxazole. Some variable susceptibility rates were observed in several studies: (70.9-71.8%) in Northern Israel (Wasseem *et al.*, 2007), 31.9 and 40% at Dakar (Jean-Marie *et al.*, 2007; Diop-Ndiaye *et al.*, 2014), 15% in Bangui (Hadiza *et al.*, 2003), 35% to Pakistan (Ahmad *et al.*, 2015). TMP-SMX resistance rates of 25-68% were also reported in others countries (Urbina *et al.*, 1989; Wylie and Koornhof, 1989). After 2011 year, a total loss of effectiveness was observed with this antibiotic, that should be explained by its no medically in use through its supply by the people in the market.

The effectiveness rates of ampicillin and amoxicillin in our study were 33% and 25% respectively. Primarily, resistance to these antibiotics by β-lactamase producing *E. coli* come from their sub-inhibitory concentrations (Moura *et al.*, 2009). Concerning amoxicillin, some studies conducted at Dakar have shown similar rates as in our study (Jean-Marie *et al.*, 2007; Diop-Ndiaye *et al.*, 2014). As for ampicillin, the study investigated the prevalence of antibiotic resistance in urinary tract infections caused by *E. coli* in children showed 53.4% of resistance rate (Ashley *et al.*, 2016) while 67% has been reported in our study and Turkey (Nisel *et al.*, 2016). Of all cases observed in 16 countries in Europe plus Canada, the *E. coli* resistance to ampicillin was 30% (Kahlmeter, 2003b).

The amoxicillin/clavulanic acid activity depends on the level of β-lactamase production by *E. coli*. In our study, it efficacy rate was 82% during all study period, more one fold higher that reported (59.3%) by (Alemu *et al.*, 2012). An opposite, Sharan reported 88% of resistance among children less of 5 years in India (Rajiv *et al.*, 2013).

A resistance rate of 86% of *E. coli* to carbenicillin was observed in our study, similar that reported (100%) on children diarrhea and swim areas on Tigris River in Baghdad city (Israa *et al.*, 2014).

Cephalosporins activity against *E. coli* has increased from first to fourth generation and the new classes were too much. Recently, the number of resistant strains to cephalosporins has increased (Ribeiro *et al.*, 2002). In our study, four antibiotics of this class were tested. Cefotaxim and ceftriaxon showed effectiveness to 100% during use years. Our results were similar those observed at Dakar (97%) and Northern Israel (98%) for each antibiotic respectively (Jean-Marie *et al.*, 2007; Urbina *et al.*, 1989).

In contrary, Sharan reported a high resistance for both antibiotics (73.5% and 73.5%) among children less of 5 years in India (Rajiv *et al.*, 2013). For cephalosporin for three years, a 50% susceptibility rate was reported in our study. Wasseem *et al.* (2007) reported effective rate of cephalexin to *E. coli* ranging between (60%-74%) in Northern Israel. About cephalixin, a high resistance rate of 80% of this antibiotic to *E. coli* was observed as reported by Datta in India (Datta *et al.*, 2004).

Severe UTI requires appropriate antimicrobial treatment preferably cephalosporins (third generation).

But, in patients with an allergy to cephalosporins, aztreonam may be used (Grabe *et al.*, 2015). Our study showed effectiveness of aztreonam to *E. coli* at 100%. This effectiveness was already seen since 2005 with 67% rate. In India, Iraq *et al.* (2015) reported 56.7% of susceptibility rate among children less than 12 years.

As for nitrofurantoin, a high effectiveness (100%) observed in our study was similar that observed in Portugal (94%) (Inês *et al.*, 2013) and India (100%) (Rajiv *et al.*, 2013). In several studies, nitrofurantoin resistance rates remained at lower levels (Arman *et al.*, 2012; Kahlmeter and Poulsen, 2012; Nisel *et al.*, 2016). The lower rate (1.3%) was reported equally in study performed among children used antibiotics in primary care by (Ashley *et al.*, 2016). Because their therapy efficacy has been demonstrated clinically and microbiologically (69% and 68%) success respectively (İşkdoğan *et al.*, 2012), nitrofuratoïn should be recommended for ambulantary treatment and the cystitis and pyelonephritis treatment in women even pregnancy (Kalpana *et al.*, 2011). It is a good alternative for uncomplicated UTI treatment when it is dispensed at appropriate dosages and suitable time intervals (Nisel *et al.*, 2016).

Based on our above results mentioned, *E. coli* susceptibility decreased until 33% for ampicillin, 30% for minocyclin, 24% for tetracyclin and 25% for
amoxicillin and fallen to 14% for carbenicillin, 0% in 2015 for TMP-SMX, remains around 75% for ciprofloxacin, while cephalosporin third generation, aztreonam and nitrofurantoin were maintained their effectiveness rate until 100%.

These results are important in medical practice in Benin. We have confirmed now that ampicillin, minocyclin, tetracyclin, amoxicillin, carbenicillin and TMP-SMX couldn’t be appropriated for treatment of UTI and that use of quinolone could exposed to resistance in 25% of cases and that third-generation cephalosporin, aztreonam and nitrofurantoin are those that offers the highest effectiveness of UTI treatment.

One limitation of this study is the low number of patients per year that did not allow a comparative analysis of the resistance of *E. coli* during the 11 years of study.

**Conclusion**

The use of drugs such as minocyclin, ampicillin, amoxicillin, carbenicillin, cephalothin, cephalaxin and trimethoprim/sulfamethoxazole does not seem appropriate for empirical treatment of UTI. These findings highlighted the importance in Benin to implement national guideline of antibiotics use and done toward medic and nurse training.

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**Author Contributions**

Tchiakpe Edmond: Study design, data collection, data analysis and writing.
Laurence Carine Yehouenou and Zahra Fall Malick: Study design, writing and reviewing.
Kpangon Amadou Arsène and Abou Abdallah Malick Diouara: Study design writing.
Keke Kpemahouton René: Final approval of the version to be submitted.
Issa Tondé: Contributed to the writing.
Honoré Sourou Bankolé and Halimatou Diop Ndiaye: Study design, data collection and writing.
Bekou Kossi Willfried and Esse Atehni Marius: Statistical analysis.
Coumba Touré Kane: Study design and data collection.

**Conflict of Interest**

There are no conflicts of interest to declare.

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