Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

# Molecular Aspects of Resistance to Antibiotic of Community *Escherichia coli* Uropathogenic Strains in Bamako

**Sidibe-Koïta M[1], Traore AM[2], Maïga AI[3], Minta DK[2], Toure-Kane CN[4], Bouh CS[4], Maïga I[1] and Gassama-Sow A[4]**

[1]*Medical Biology and Hospital Hygiene Laboratory Bacteriological-Virology Laboratory of Point G, Bamako-Mali.*

[2]*Infectious Diseases Department of the University Hospital Center Point G, Bamako-Mali.*

[3]*Bacteriological-Virology Laboratory of the University Hospital Gabriel Toure, Bamako-Mali.*

[4]*Bacteriological-Virology Laboratory of the University Hospital Center LeDantec of Dakar-Senegal.*

***Correspondence:**
Abdoulaye Mamadou Traoré, Infectious Diseases Department of the University Hospital Center Point G, Bamako-Mali, E-mail: amtraore2008@gmail.com

**Received:** 14 November 2019; **Accepted:** 05 December 2019

**Citation:** Sidibe-Koïta M, Traore AM, Maïga AI, et al. Molecular Aspects of Resistance to Antibiotic of Community *Escherichia coli* Uropathogenic Strains in Bamako. Microbiol Infect Dis. 2019; 3(4): 1-5.

## ABSTRACT

*The aim of this study was to determine the resistance phenotypes and genes involved in antibiotic resistance of community uropathogenic E. coli strains in Bamako. E. coli strains were isolated from the urine from September 2011 to June 2012 of external patients who were seen in specialized department of CHU, research of resistance support (integrons and resistance genes) were performed at the Experimental Bacteriology Unit at the Institute Pasteur of Dakar (Senegal). During the study, we have isolated 29 strains of E. coli, the majority produced beta-lactamase extended-spectrum (55.17%).*

*The resistance was high to amoxicillin, third generation cephalosporins including ESBL (55%), sulfamethoxazole-trimethoprim and fluoroquinolones.*

*Genetic supports detected were $bla_{TEM1}$ (72%), $bla_{SHV1}$ (38%), $bla_{OXA1}$ (52%), $bla_{CTXM1}$ et $bla_{CMY1}$ (44%), $bla_{CTXM9}$ (13%), QnrB (4%) et QnrS (88%). The majority of ESBL-producing strains presented the QnrS gene (88%). Integrons Class 1 (intl 1) were found in 31% of strains and class 2 integrons (intl 2) at 7%. No class 3 integron was detected.The QnrB gene was found in only a single strain. No strain has presented the QnrA gene.*

*This growing increase in resistance to various antibiotics, confirmed by the presence of plasmid genes dictate the strengthening of the surveillance of resistance in our country, through good practice in antibiotic therapy in the hospital and in the community. We must establish a monitoring network antibiotic involving private practice physicians, hospitals and laboratories.*

## Keywords

Escherichia coli, Resistance, Antibiotics, Community urinary infections.

## Introduction

The urinary tract is the second site of community bacterial infection after the respiratory one and motivates the prescription of antibiotics in clinical practice [1]. Escherichia coli (*E. coli*) is the major bacterial specie involved in infections of the urinary tract [2,3]. Fluoroquinolones (FQ) and β-lactams (BL) are molecules of choice for treating urinary tract infections [3].

The increase in bacterial resistance to many antibiotics has become a worldwide public health problem [4]. In *E. coli*, the main mechanism of resistance to beta-lactam antibiotics is enzymatic through production of beta-lactamases [5].

The emergence of beta-lactamase with extended spectrum (ESBLs)

and their global spreading represent a significant public health threat. For the quinolones, the genetic support of the resistance can have a plasmid feature (QnrA, QnrB and QnrS) for several enteric bacteria species [6,7].

A more rational use of antibiotics, both in community and hospitals requires an updated knowledge of resistance phenotypes and genes involved in order to enable better management of infections caused by these bacteria.

In Mali, few studies have been done on the molecular aspect of the resistance of *E. coli* strains to antibiotic. The overall objective of this work was to determine the resistance phenotypes and genes involved in antibiotic resistance of community uropathogenic *E. coli* strains in Bamako. It was more specifically:

- To isolate and identify the community uropathogenic strains of *E. coli* in Bamako;
- To test their sensibility to antibiotics to determine the different resistance phenotypes;
- To search the genetic supports involved in the resistance to quinolones and beta-lactams.

## Materials and Methods

### Patients

The study was prospective, extending from September 2, 2011 to November 30, 2012. Patient recruitment and identification of *E. coli* strains were performed in the Medical Biology and Hospital Hygiene Laboratory of the University Hospital Center (CHU) of Point G in Bamako. Re-identification, implementation of sensibility tests to antibiotics and research of resistance supports (integrons and resistance genes) were performed at the Experimental Bacteriology Unit at the Pasteur Institute of Dakar (Senegal).

### Inclusion and exclusion criteria

*E. coli* strains were isolated from the urine of external patients who were seen in specialized department of CHU.

*E. coli* isolated from urine of hospitalized patients and other pathogens were not considered for this study.

### Methods

Investigation sheets were conceived to collect socio-demographic information (age, gender, origin, marital status, profession), medical history (antibiotics' swallowing or not, hospitalization history) and in women (existence or not of a pregnancy). The *E. coli* strains isolated in Bamako were stored at -80°C in brain heart broth at 5% glycerol and then carried to the Experimental Bacteriology Unit at the Pasteur Institute of Dakar (Senegal) where we proceeded to re-identifying the strains, to sensibility tests to antibiotics, and to research of resistance supports (integrons and resistance genes). About the study of the sensibility to antibiotics, we had on susceptibility testing for each identified bacteria, according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (CA-SFM, 2012 edition). To re-identify, the strains in brain heart broth with 5% glycerol were re- isolated on an agar - bromo- cresol purple (BCP) and incubated at 37°C (98.6°F) for 18 to 24 hours. Confirmation for *E. coli* was made on the basis of cultural and biochemical features using the conventional gallery or the API 20E (Biomerieux, France). As controls we used strains of *Salmonella* Concord 05-5343: *TEM, QnrA*; *Salmonella* Concord 07-670: *TEM, SHV*, Integron classe 1 (*IntI 1*); *Enterobacter cloacae* AME: *QnrS et Salmonella* Havana 07-319: *QnrB*.

About the DNA extraction, it was made with the QIAGEN kit (Qiamp DNA Mini Kit Cat 51304 Qiagen) (after isolating the strains on TCS agar (tryptic soy) 18-24h).

Research of resistance genes and integrons by gene amplification
The genes of resistance to antibiotics and integrons were searched using classic PCR (Polymerase Chain Reaction) with specific primers for each resistance gene or integron class. The desired genes were: $bla_{TEM1}$, $bla_{SHV1}$, $bla_{OXA1}$ that encode resistance to penicillins, $bla_{CTXM1}$ genes $bla_{CTXM9}$, $bla_{CMY1}$ that encode resistance to third-generation cephalosporins and QnrA genes QnrB, QnrS that encode resistance to quinolones. The desired integrons were: class 1 integrons (intl 1), class 2 integrons (intl 2) and class 3 integrons (intl 3).

## Results

During the study, we isolated 29 strains of *E. coli* on what we looked for resistance phenotypes. Figure 1 summarizes the antibiotic resistance profile.

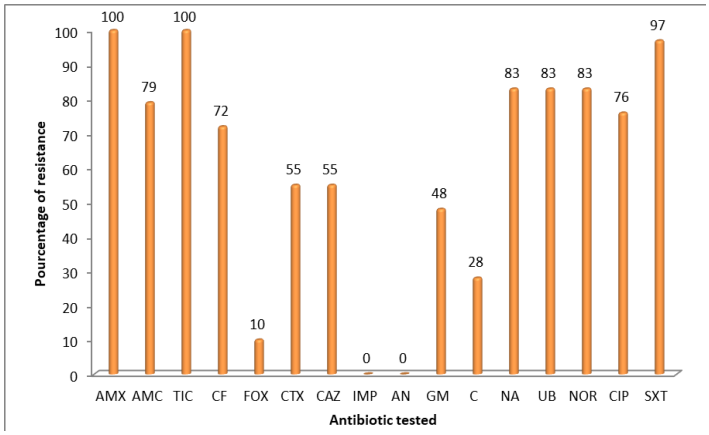


**Figure 1:** Percentage of resistance of *E. coli* strains regarding the tested antibiotics.
AMX: Amoxicillin (25µg); AMC: Amoxicillin/clavulanic acid (20/10µg); TIC: Ticarcilline (75µg); CF: Cefalotin (30µg); FOX: Cefoxitin (30µg); CTX: Cefotaxime (30µg); CAZ: Ceftazidime (30µg); IMP: Imipenème (10µg); AN: Amikacin (30µg); GM: Gentamicin (10UI-15µg); C: Chloramphenicol (30µg); NA: Nalidixic acid (30µg); UB: Flumequin (30µg); NOR: Norfloxacin (5µg); CIP: Ciprofloxacin (5µg); SXT: Trimethoprin/Sulfaméthoxazol (1,25/23,75µg).

### The phenotypes of resistance to β-lactams

Analysis of the antibiogram results allowed us to identify four groups of resistance phenotypes to beta-lactams: PBN (penicillinase at low level), TRI (TEM resistant to inhibitors), PHN (high-level

penicillinase), BLSE (beta-lactamase extended spectrum). Most of the isolated strains of *E. coli* produced beta-lactamase extended-spectrum (55.17%), followed by those producing penicillinase at low level (17.24%), penicillinase at high level (17.24 %) and those producing resistant to inhibitors or TRI (10.35%). However, none of the strains presented a sensitive or wild-type. The percentages of the different phenotypes are represented below in Figure 2.

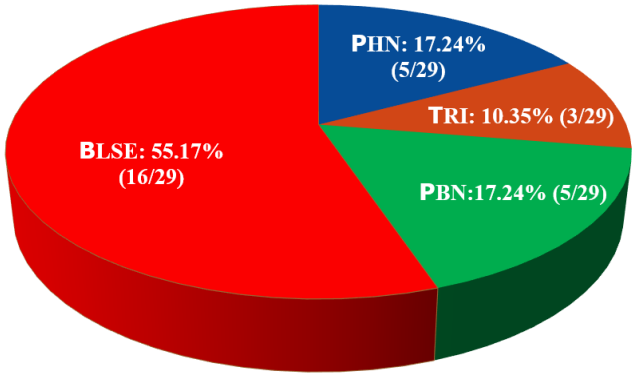


**Figure 2:** Distribution of strains according to the resistant phenotypes to beta-lactams.

## Quinolone resistance phenotypes

The results of the antibiogram allowed us to identify four groups of resistance phenotype to quinolones (Table 1). Most of the tested strains, 76% (group III) was resistant to first-generation of quinolones (nalidixic acid, flumequine) and to fluoroquinolones (ciprofloxacin and norfloxacin). The phenotypes of group I and group II were poorly represented (7%). Only 17% of the strains were of sensitive phenotype (Table 1).

| Groups | Phenotypes | Number of strains (%) |
|---|---|---|
| Group I | NALR UBR NORI CIPS | 1 (3.5) |
| Group II | NALR UBR NORR CIPS | 1 (3.5) |
| Group III | NALR UBR NORR CIPR | 22 (76) |
| Total | | 24 (83) |

**Table 1:** Distribution of quinolone resistance phenotypes.

## Supports of resistance (integron and resistance genes)

The search for resistance genes found that 72 % of the strains carried the blaTEM1 gene against 38% of blaSHV1 and 52% blaOXA1.

Amongst the 16 strains that produce BLSE, 7 (44%) were carrying the blaTEM1gene, 2 (13%) the blaCTXM9 gene and 7 (44%) the blaCMY1 gene.

The Qnr gene was found on 22 out of the 24 strains resistant to quinolones et fluoroquinolones which is 92% of the studies strains. The QnrS gene was mostly found in the strains (88%), followed by QnB (4%). No QnrA gene was found.

About the integrons, we found class 1 integrons (intI 1) in 31% of the strains and class 2 integrons (intI 2) in 7% of the strains; however, no class 3 integron was (intI 3) found. The caracterisation of the ontegrons was not done.

Phenotypes and genetic determinants of resistance to antibiotics

The genetic supports of penicillinase phenotypes were blaTEM1 genes found in 92% of strains, blaSHV1 (31% of the strains) and (blaOXA1) in 54% of the strains. Among these strains, four presented class 1 integrons in addition to the listed genes.

A penicillinase producing strain at low level was not wearing any of the desired genes, but the class 1 integrons (intl gene 1) (Table 2).

Another strain presented class 2 integrons (intl gene 2) in addition to blaTEM1, blaSHV1 and blaOXA1 genes, of the 13 non-ESBL-producing strains, 5 were sensitive to the quinolones and fluoroquinolones tested and of the 8 resistant strains, only one was not wearing a QnrS gene. The resistance phenotypes and their genetic determinants are shown in Table 2.

| Number of strains | Resistance phenotypes | Genetic determinants of the resistance | | |
|---|---|---|---|---|
| | | Gene IntI1 | Genes of resistance to betalactams | Genes of resistance to quinolones |
| N=1 | $AMX^{R}TIC^{R}$ $NA^{R}UB^{R}NOR^{R}SXT^{R}$ | intI 1 | | *QnrS* |
| N=1 | | intI 1 | $bla_{TEM1}$, $bla_{SHV1}$, $bla_{OXA1}$ | - |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}C$-$F^{R}SXT^{R}$ | intI 1 | $bla_{TEM1}$ | - |
| N=1 | | intI 1 | $bla_{TEM1}$, $bla_{OXA1}$ | |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}CF^{R}$ $NA^{R}UB^{R}NOR^{R}CIP^{R}SXT^{R}$ | intI 1 | $bla_{TEM1}$ | *QnrS* |
| N=1 | $AMX^{R}TIC^{R}$ $NA^{R}UB^{R}NOR^{R}CIPRSX$-$T^{R}$ | intI 2 | $bla_{TEM1}$, $bla_{SHV1}$, $bla_{OXA1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1}$, $bla_{SHV1}$ | *QnrS* |
| N=1 | $AMX^{R}TIC^{R}SXT^{R}C^{R}$ | - | $bla_{TEM1}$ | - |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}SXT^{R}$ | - | $bla_{TEM1}$ | - |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}$ $NA^{R}UB^{R}NOR^{R}CIP^{R}SXT^{R}$ | - | $bla_{TEM1}$, $bla_{OXA1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1}$, $bla_{SHV1}$, $bla_{OXA1}$ | *QnrS* |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}C$-$F^{R}SXT^{R}$ | - | $bla_{TEM1}$ | - |
| N=1 | $AMX^{R}AMC^{R}TIC^{R}CF^{R}$ $NA^{R}UB^{R}NOR^{R}CIP^{R}SX$-$T^{R}GM^{R}$ | - | $bla_{TEM1}$, $bla_{OXA1}$ | - |

**Table 2:** Antibiotic resistance phenotypes and genetic determinants in non-ESBL-producing strains of *E. coli*.

The ESBL-producing strains, presented multiresistant phenotypes towards the tested antibiotics. Indeed, all these strains were resistant to the quinolones and fluoroquinolones tested, in addition, they were resistant to other antibiotics such as gentamicin, sulfamethoxazole / trimethoprim.

Genes found in these strains were: blaCTXM1 (44%), blaCTXM 9 (13%) and blaCMY1 (44%); 56% of these strains presented the blaTEM1 gene, 44% the blaSHV1 gene and 50% the blaOXA1

one.

Two of ESBL-producing strains presented only class 1 integrons (intl gene 1). The majority of ESBL-producing strains presented the QnrS gene (88%). The QnrB gene was found in only a single strain. No strain presented the QnrA gene (Table 3).

| Number of strains | Resistance phenotypes | Genetic determinants of the resistance | | |
|---|---|---|---|---|
| | | Genes intI | Genes of resistance to betalactams | Genes of resistance to quinolones |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RCAZ^R$ $NA^RUB^RNOR^R$ $CIP^RSXT^R$ | intI 1 | $bla_{OXA1,}$ $bla_{CTXM1}$ | *QnrS* |
| N=1 | | intI 1 | - | *QnrS* |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RCAZ^R$ $NA^RUB^RNOR^RCIP^RSX$-$T^RGM^R$ | intI 1 | - | - |
| N=1 | | intI 1 | $bla_{OXA1}$ | *QnrS* |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RCAZ^R$ $NA^RUB^RNOR^RCIP$-$^RGM^R$ | intI 2 | $bla_{TEM1,}$ $bla_{SHV1}$ $bla_{CMY1}$ | *QnrS, QnrB* |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RFOX^RCAZ^R$ $NA^RUB^RNORR$-$CIP^RSXT^RGM^R$ | - | $bla_{TEM1,}$ $bla_{SHV1}$ $bla_{CTXM9,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1,}$ $bla_{SHV1}$ $bla_{CTXM1,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1,}$ $bla_{OXA1}$ $bla_{CTXM1}$ | *QnrS* |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RCAZ^R$ $NA^RUB^RNOR^RCIP^RSX$-$T^RGM^R$ | - | $bla_{TEM1,}$ $bla_{SHV1}$ $bla_{OXA1,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{OXA1,}$ $bla_{SHV1}$ $bla_{CTXM1,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1,}$ $bla_{CTXM1}$ | *QnrS* |
| N=1 | | - | $bla_{OXA1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1,}$ $bla_{SHV1,}$ $bla_{CTXM9,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{OXA1,}$ $bla_{CTXM1}$ | - |
| N=1 | $AM^RAMC^RTIC^RC$-$F^RCTX^RCAZ^R$ $NA^RUB^RNOR^R$ $CIP^RSXT^R$ | - | $bla_{TEM1,}$ $bla_{OXA1}$ $bla_{CTXM1,}$ $bla_{CMY1}$ | *QnrS* |
| N=1 | | - | $bla_{TEM1,}$ $bla_{SHV1}$ | *QnrS* |

**Table 3:** Phenotypes and genetic determinants of resistance in ESBL-producing strains of *E. coli*.

- = Absence of genes (or integrons).

## Discussion

*E. coli* was the most frequently isolated germ in the urinary tract infections during our study period. Other authors have made the same observations [2,8].

Our study allowed us to indicate that the percentage of resistance of *E. coli* community ESBL-producing strains was very high for a lot of antibiotics. In Bamako, the rate of *E. coli* ESBL-producing strains in the community was 12% in 2004 against 15.4% in 2005 and 19.3% in 2006 [9]; this indicates its net increasing.

High resistance rates of *E. coli* to amoxicillin + clavulanic acid and to Cotrimoxazol (trimethoprim/sulfamethoxazol) widely justify their non-use in first-line treatment of urinary infection. We identified the blaTEM1 genes (72%), blaOXA1 (52%) and blaSHV1 (38%) as predominantly associated with resistance to aminopenicillins.

In our context, cephalosporins are a good remedy for the treatment of urinary infections. However, we found a large proportion of resistance (55-72%). All strains were resistant to C3G produced ESBL and carried blaCTXM1 (44%), blaCTXM9 (13%) and blaCMY1 (44%) genes. The sequencing should allow to determine the type of CTX. A previous study in the same university hospital center in 2007 showed that ESBL-producing *E. coli* strains isolated in Bamako presented CTXM-15 genes (89%), CTXM-14 (10.1%) and SHV-5 (0.9%) [9].

In Mali, as in other African countries, quinolones (ciprofloxacin and norfloxacin) are molecules commonly used to treat urinary tract infections [10]. In our study, the proportion of resistance to ciprofloxacin (76%) and norfloxacin (83%) remains high. For the molecular markers of resistance, 88% of strains resistant to quinolones carried the *QnrS* gene and the association QNR-ESBL was found in 67% of strains. This association "QNR-ESBL" was found in other studies. Ivory Coast prevalence "QNR-ESBL" was 31% in *E. coli* [11]. In Morocco, Bouchakour et al. had found the Qnr gene in 18.7% of strains of *E. coli* ESBL [12].

Previously, no study had been conducted in Mali on integrons research among uropathogenic *E. coli* strains. In this way, ours was a pilot study. Integrons were also found in this study. Among the three searched integrons, classes, class 1 integrons (intl 1 gene) were mostly found in 31% of strains and class 2 integrons (intl 2 gene) in 7%. Other authors had described that in enterobacteria, Class 1 integrons were more common, followed by class 2 [15]. We have not found class 3 integrons and coexistence of class 1 and class 2 in our study.

Overall, resistance increasing factors are unclear. However, some authors believe that the consumption of inappropriate antibiotics is a major risk of resistance; others have correlated that with overall high use of this antibiotic family in the community [8,13,14].

## Conclusion

This study reveals a high rate of *E. coli* resistance to the most prescribed antibiotics (amoxicillin + clavulanic acid amoxcilline, fluoroquinolones, cephalosporins, trimethoprim-sulfamethoxazole) in the treatment of urinary tract infections. Antibiotics that keep good sensitivity are those that are not accessible (not available or no AMM). This growing increase in resistance to various antibiotics, confirmed by the presence of plasmid genes dictate the strengthening of the surveillance of this resistance in our country, through good practices in antibiotic therapy in the hospital and in the community (by promoting health education in order to reduce the overuse of antibiotics). It is also necessary to strengthen the fight against illegal drug sales.

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