# Bacteriological profile and resistance of *Escherichia coli to* beta-lactam for patients consulted at Douala Laquintinie Hospital

## ABSTRACT

Enterobacteriaceae are the most common causes of community-acquired and nosocomial infections. They are usually treated with beta-lactam antibiotics, i.e., penicillins, broad-spectrum cephalosporins, and carbapenems (Imipenem, meropenem, ertapenem). In order to evaluate the resistance profile of *Escherichia coli* (*E. coli*) to beta-lactam antibiotics, a 3-year retrospective study was carried out in the medical biology laboratory of the Laquintinie Hospital in Douala. The aim was to compare the behavior of *Escherichia coli* bacteria to penicillins, carbapenems, and cephalosporins. In order to achieve this objective, we used the results obtained from E. coli strains isolated from urine, cervico-vaginal fluid, puncture fluid, pleural fluid and pus samples. Identification was confirmed usingthe API 20 E *Enterobacteriaceae* system of biochemical testingand the VITEK 2 system. The VITEK 2 system was used to perform the antibiogram. Statistical analyses obtained from GraphPad Prim V 5.0 software allowed us to perform tests such as ANOVA, Kruskal-Wallis test, and Spearman correlations. Preliminary results show that *E. coli* bacteria are highly resistant to penicillins and significantly susceptible to cephalosporins of all generations if they are not ESBL (extended-spectrum beta-lactamases). Data on carbapenem behavior show less resistance and moderate susceptibility.

**Keywords**: Bacterial resistance, antibiotics, *Escherichia coli*, beta-lactams, Douala

## 1. Introduction

*Escherichia coli ,* a gram-negative bacillus of the Enterobacteriaceae family and a commensal of the gastrointestinal tract (108 per gram of feces), is the most frequently isolated uropathogenic bacterium in uncomplicated UTIs (70-95%) [1].

Other bacteria isolated include other enterobacteria (*Klebsiella spp., Proteus spp.).* The bacterial epidemiology of complicated UTIs is more varied; E. coli remains the most common bacterium (50%), along with *Klebsiella spp.* and Proteus spp. but also *Pseudomonas aeruginosa,* enterococci and yeasts[2]. *Escherichia coli* is a bacterium commonly found in the digestive tract of humans and warm-blooded organisms. Most strains are harmless. Some, however, can cause severe food poisoning that can lead to serious illnesses, including urinary tract infections [3].

Urinary tract infections are a major public health problem. According to the World Health Organisation (WHO), *E. coli*urinary tract infections are the most common in hospitals and communities. The choice of an antibiotic, regardless of the species or resistance mechanism involved, must meet requirements to ensure clinical and microbiological success. In addition to the in-vitro sensitivity of the species concerned to the chosen antibiotic, the latter must have the best possible diffusion allowing it to reach the infectious site at sufficient tissue concentrations compared to the minimum inhibitory concentration

(MIC) of the isolated bacterium [4]. The beta-lactams are effective; they act by inhibiting the PLP (penicillin-binding protein) enzymes essential for the final stage of peptidoglycan synthesis [5]. In recent years, there has been an increase in antibiotic resistance to UTIs. The emergence of Broad Spectrum Beta-Lactamase (ESBL) secreting Enterobacteriaceae is increasingly prevalent [6]. It appears important to evaluate the behavior of antibiotics against *E. coli* in patients consulting the Laquintinie Hospital of Douala for better therapeutic management. This approach allows us to characterize the bacteriological resistance profile of *E. coli* to beta-lactam antibiotics in this hospital, particularly penicillins, carbapenems, and cephalosporins.

## 2. Materials and methods

### Design of the study

This is a retrospective descriptive study. The data for this study were obtained from bacterial strains of Escherichia coli isolated and identified from patients attending the bacteriology laboratory of Laquintinie Hospital in Douala during the period from 1 January to 31 December 2019.

### Sample Collection and Bacterial Isolation

The different *E. coli* strains tested had been isolated from urine, cervico-vaginal fluid, puncture fluid, pleural fluid and pus samples. their identification had been made through their morphological and biochemical characteristics (Gram-negative bacilli, oxidase-negative) completed using the API 20 E *Enterobacteriaceae* system of biochemical testing (BioMérieux, France) and The VITEK 2 automated system (BioMérieux, France). A total of 2332 E. coli strains had been isolated and identified during 2019.

### Antimicrobial Susceptibility Testing

The VITEK 2 automated system (BioMérieux, France) was used with antimicrobial susceptibility testing (AST), respectively, of gram-negative bacilli. The antimicrobial agents tested were amikacin, amoxicillin-clavulanic acid, ampicillin, cefalotin, cefepime, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, meropenem, nitrofurantoin, norfloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, tobramycin, and trimethoprim-sulfamethoxazole.

### Statistical analysis

Statistical analysis was carried out using different software packages, initially using Excel from office 2010 as the spreadsheet. The data was then exported to analysis software such as Graph Pad Prism V.5.0 to perform Kruskal Wallis tests and spearman correlations. A value of $p < 0.05$ were considered statistically significant.

## 3. Results

### Different sampling sites

During the study period, 2332 bacterial strains of *E. coli* were isolated, identified, and classified into three clinical categories: two susceptible categories (standard dose susceptible (**S**) and high exposure susceptible (**I**) and one resistant category (**R**) according to the recommendations of the Antibiotic Committee of the French Society of Microbiology (CASFM) V1.0 Mai 2022. The strains isolated were mainly from urine (59.61%), pus (19.47%), pleural fluid (9.01%), puncture fluid (4.03%), and finally, cervical-vaginal swab (7.89%), as shown in Table 1. Resistance was found in 48.8% of cases, with 2.3% in intermediate cases and 48.7% in sensitive cases. Of the resistant germs isolated, more than half were found in the urine and more than a quarter in the pus. Among the susceptible germs, 61.16% were found in urine and 11.07% in pus. Of the highly exposed susceptible germs, 53.70% were found in urine and 29.63% in pus. In general, urine and pus were the specimens in which a high frequency of resistance was observed.

**Table 1.** Antimicrobial susceptibility profile of the Romanian fresh-cheese-origin *E. coli* strains tested with the VITEK®

| **sample** | **Susceptibility Test Result of 2332 Strains (n/%)** | | | **Total** |
|---|---|---|---|---|
| | **R** | **I** | **S** | |
| Urine | 665 (58.3) | 29 (53.7) | 696 (61.2) | 1390 |
| Cervical-vaginal swab | 49 (4.3) | 3 (5.6) | 132(11.6) | 184 |
| Puncturefluid | 34 (3) | 3 (5.6) | 57 (5) | 94 |
| Pleural fluid | 80 (7) | 3 (5.6) | 127(11.2) | 210 |
| Pus | 312 (27.4) | 16 (29.6) | 126 (11.1) | 454 |
| Total | **1140** | **54** | **1138** | **2332** |
| P Value | **0.0003** | **0.005** | **<0.001** | |

**Description: R:** resistant category; **S:** standard dose susceptible; **I:** high exposure susceptible.

**The behavior of germs towards the different antibiotics tested in different samples.**

The evaluation of the distribution of the germ's behavior towards the different antibiotics tested shows a high resistance of the germ isolated in the urine to penicillins (17.1%), carbapenems (2.6%), and cephalosporins (28.1%). The *Escherichia coli* germs found in pus also showed high resistance to penicillins (26.3%), carbapenems (7.6%), and cephalosporins (28.1%). The behavior of *Escherichia coli* in specimen media other than urine and pus showed less than 10% resistance to the three different styles of antibiotics, as did cervical-vaginal specimens (CVS), which showed 1.2% resistance to penicillins, 0.6% to carbapenems and 1.7% to cephalosporins. Concerning the sensitivities, we observe a high sensitivity for *E. coli* strains isolated in urine, notably for cephalosporins (29.8%) and carbapenems (13.2%).

**Table 2.The behavior of the different antibiotics at the sampling sites**

| **sample** | **Susceptibility Test Result of 2332 Strains (n/%)** | | | | | | | | | **P value** |
|---|---|---|---|---|---|---|---|---|---|---|
| | R | | | I | | | S | | | |
| | **Rp** | **Rc** | **Rce** | **Ip** | **Ic** | **Ice** | **Sp** | **Sc** | **Sce** | |
| Urine | 17.1 | 2.6 | 28.1 | 0.4 | 0.2 | 1.5 | 7.1 | 13,2 | 29.8 | **0.006** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cervico-vaginal swab | 1.2 | 0.6 | 1.7 | 0.0 | 0.0 | 0.2 | 0.9 | 2,2 | 6.3 |
| PunctureLiquid | 1.4 | 0.1 | 0.9 | 0.0 | 0.1 | 0.1 | 0.9 | 1,8 | 1.4 |
| Pleural fluid | 0.9 | 0.1 | 4.7 | 0.0 | 0.2 | 0.0 | 0.3 | 2.4 | 6.4 |
| Pus | 5.7 | 4.0 | 12.7 | 0.1 | 0.1 | 0.9 | 0.2 | 3.7 | 5.1 |

**Description: Rp**: Resistant to penicillins; **Rc**: Resistant to carbapenems; **Rce**: Resistant to cephalosporins; **Ip**: Susceptible at a high exposure to penicillins; **Ic**: Susceptible at a high exposure to carbapenems; **Ice**: Susceptible at a high exposure to cephalosporins.

### Distribution of antibiotic behavior on *Escherichia coli*

Figure 1 below shows the behavior of the various antibiotics against *E. coli.* It can be seen that, in general, cephalosporins were more active on the different strains of *Escherichia coli* in terms of resistance (more than 18%) with a significant P value (P Value=0.016) and terms of sensitivity (more than 20%) with significant P values (0.0017). Carbapenems (more than 7%) were more sensitive than penicillins (less than 5%) and consequently less resistant than penicillins (more than 10%).

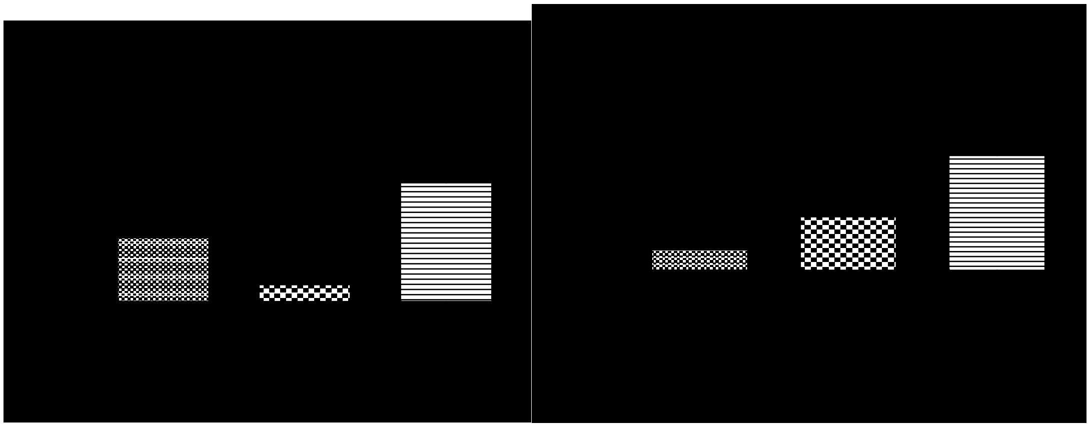

**Figure** 1**: Distribution of the behavior of different antibiotics (Betalactamins) towards** *Escherichia coli*

**Description**: A: Sensible; B: Resistances; Significant < 0,005 (Kruskal-Wallis and Friedman statistic via ANOVA Analysis); **Rp**: Resistant to penicillin's; **Rc**: Resistant to carbapenems; **Rce**: Resistant to cephalosporins; **Ip**: Susceptible at a high exposure to penicillin's; **Ic**: Susceptible at a high exposure to carbapenems; **Ice**: Susceptible at a high exposure to cephalosporins.

### Bacteriological profile of *Escherichia coli* and penicillin resistance

Amongst the Beta-lactam antibiotics tested for antibiotic susceptibility, there was a resistance of over 8.58% compared to a sensitivity of 1.8% for ampicillin and less than 1% for oxacillin and ticarcillin.

**Table 3.** The behavior of *Escherichia coli* towardspenicillin's

| **Antimicrobial** | **Susceptibility Test Result of 2332 Strains (n/%)** | | |
|---|---|---|---|
| | R | I | S |
| AMOX | 2 (0.7) | 0 (0.0) | 0 (0.0) |
| AMP | 200 (74.6) | 0(0.0) | 24 (8.9) |

|  |  |  |  |
|---|---|---|---|
| OXA | 16 (5.9) | 1 (0.37) | 0 (0.0) |
| TIC | 22 (8.2) | 0(0.0) | 3 (1.1) |
| P value |  | **0.0276** |  |

**Description**: **R:** resistant category; **S:** standard dose susceptible; **I:** high exposure susceptible; **AMOX**: Amoxicillin; **AMP**: Ampicillin; **OXA**: Oxacillin; **TIC**: Ticarcillin

### Bacteriological profile of *Escherichia coli* and carbapenem and monobactam resistance

Several carbapenem and monobactam antibiotics were tested on *Escherichia coli* during this period. Almost 1% resistance was observed with imipenem and less than 1% with Meropenem. Meropenem showed rather high sensitivities compared to the other, up to more than 11%. A sensitivity of more than 2% was also observed with imipenem. The sensitivity of Azthreonam was the lowest observed, with less than 1%.

**Table 4.**Behavior towards carbapenems and Azthreonam

| **Antimicrobial** | **Susceptibility Test Result of 2332 Strains (n/%)** |  |  |
|---|---|---|---|
|  | **R** | **I** | **S** |
| ATM | 0 (0.0) | 8 (1.9) | 12 (2.9) |
| IMI | 24 (5.9) | 0 (0.0) | 68 (16.8) |
| MEM | 20 (4.9) | 0 (0.0) | 272 (67.3) |
| P value |  | **0.0106** |  |

**Description**: *Significant < 0.005 (Friedman test statistic); **R:** resistant category; **S:** standard dose susceptible; **I:** high exposure susceptible**;** **ATM**: Azthreonam; **IMI**: Imipenem; **MEM**: Meropenem.

### Bacteriological profile of *Escherichia coli* and cephalosporin resistance

Several cephalosporin antibiotics were tested, with other resistance observed, for other cephalosporin antibiotics less than 1%. Compared to beta-lactam sensitivity in general.

**Table 5.**Behavior towards cephalosporins

| **Antimicrobial** | **Susceptibility Test Result of 2332 Strains (n/%)** |  |  |
|---|---|---|---|
|  | **R** | **I** | **S** |
| CAZ | 66(8.8) | 12(1.6) | 52(6.9) |
| CFM | 64(8.5) | 6(0.8) | 40(5.3) |
| CMX | 32(4.7) | 0(0.0) | 12(1.6) |
| CPD | 76(10.1) | 2(0.2) | 78(10.4) |
| CRO | 78(10.4) | 2(0.2) | 74(9.8) |
| CTX | 30(4) | 0(0.0) | 64(8.5) |
| FIX | 34(4.5) | 0(0.0) | 28(3.7) |
| P value |  | **0.0012** |  |

**Description** : *Significant< 0.005 (Kruskal-Wallis statistic) ;**R :**resistantcategory ;**S :** standard dose susceptible ;**I :** high exposure susceptible ; **CAZ** :Ceftazidime; **CFM**: Ceftaroline; **CXM**: Cefuroxime; **CPD**: Cefpodoxime; **CRO**: Ceftriazone; **CTX**: Cefotaxime; **FIX**: Cefixime.

### Correlational analysis of the behavioral dynamics of different beta-lactam antibiotics *against E. coli strains.*

After observing the behavior of the different classes of beta-lactam antibiotics, it was important to establish a correlation to test a potential increase in sensitivity or a potential decrease in resistance effects of the latter.

### RESISTANCE

A strong (0.9) significant correlation (P value=0.0045) on the possibility of E. coli resistance was obtained with a combination of penicillins and cephalosporins. Negative and non-significant correlations were obtained with the other antibiotic combinations.

**Table 6.**Correlation of resistance between different antibiotics

| | **Penicillins** | **Carbapenems** | **Cephalosporins** |
|---|---|---|---|
| | **correlation coefficients** | | |
| **Penicillin** | | -0.36 | 0.90 |
| **Carbapenem** | -0.36 | | -0.13 |
| **Cephalosporin** | 0.90 | -0.13 | |
| | **P value** | | |
| **Penicillin** | | 0.35 | 0.0045 |
| **Carbapenem** | 0.35 | | 0.75 |
| **Cephalosporin** | 0.0045 | 0.75 | |

### SENSITIVITY

The comparison of the action of the different antibiotics about their capacity to increase susceptibility when combined shows no correlation between the different combinatory modes. Furthermore, all these combinations were non-significant.

**Table 7.Correlation on sensitivity between different antibiotics**

| | **Penicillins** | **Carbapenems** | **Cephalosporins** |
|---|---|---|---|
| | **correlation coefficients** | | |
| **Cephalosporins** | | -0.14 | -0.50 |
| **Carbapenems** | -0.14 | | -0.07 |
| **Penicillins** | -0.50 | -0.07 | |
| | **P value** | | |
| **Cephalosporins** | | 0.73 | 0.19 |
| **Carbapenems** | 0.73 | | 0.86 |
| **Penicillins** | 0.19 | 0.86 | |

## DISCUSSION

Based on the characterization of the bacteriological profile and resistance of *Escherichia coli* to beta-lactam in patients consulting the medical biology laboratory of the Laquintinie Hospital in Douala, this study enabled us to obtain varied results. The analyses were carried out in various samples and showed that among the 2332 non-repetitive bacterial strains isolated, the majority of the latter came from urine (59.61%). This can be explained by the fact that *E. coli* are normally harmless intestinal bacteria for humans when they remain confined to the digestive tract. [7]. The preponderance of *Escherichia coli* in urine has been found by several authors, as presented by Cheung and colleagues in 2020[8] and Wilson and collaborateurs[9]. Given the ubiquity of E. coli, it was also found in various other samples, such as pus (19.47%), data that is consistent with the work of Serraino and colleagues in 2018, who found *E. coli* at a frequency of 26.5% in pyogenic liver abscesses (PAA) [10]. In this study, *E. coli* was also isolated from pleural fluid (9. 01%) as demonstrated by Cartelle and colleagues, by isolating a clinical strain of *E. coli* from pleural fluid with high levels of resistance to cefotaxime, ceftazidime, and aztreonam has a novel CTX-M gene (bla(CTX-M-32)) whose amino acid sequence differs from that of CTX-M-1 by a single Asp240-Gly substitution [11]. The strains found in the puncture fluid (4.03%) corroborated with the ebongue and collaborators data obtained at the Douala General Hospital in 2015, CERVICAL-VAGINAL SWAB (7.89%)[12]. The resistance observed was more in urine, with a rate for penicillins of 17.1%; this could be due to the location of the bacteria in the digestive tract. This observation is consistent with data obtained by Vazouras and colleagues in 2020, showing that *E. coli* was highly resistant to ampicillin [13]. For carbapenems, resistances have been observed; these resistances to carbapenems are particularly notable in Gram-negative bacilli species; this could be since these species naturally have relatively low transmembrane diffusion coefficients to beta-lactams as presented by Nordmann Patrice in 2010[14]. Carbapenems are mainly prescribed for the treatment of enterobacterial infections of nosocomial and, more rarely, community origin. Some publications show the extension of this resistance in enterobacteria: it is no longer restricted to certain regions of the world and now affects species that may be typically community-acquired, as is E. coli [15]. The cephalosporin susceptibility observed against *E. coli* is believed to be due to the production of CTX-M ESBLs, CTX-M ESBLs, and CMY-type AmpC enzymes and an absence of CTX-M-15 ESBL production as presented by Dhanji and colleagues in 2010 [16].

## CONCLUSION

This retrospective study of 2332 samples were initiated to evaluate the resistance profile of *E. coli* to Beta-lactam antibiotics, including penicillins, carbapenems, and cephalosporins. In general, cephalosporins were more active on the different strains of *Escherichia coli* in terms of resistance and sensitivity. Carbapenems were more sensitive than penicillins and, therefore, less resistant than penicillins. However, *E. coli* had higher resistance to penicillins, and carbapenems and less than cephalosporins, with low susceptibility, compared to cephalosporins and carbapenems. Some cephalosporins were intermediate, showing a strong ability to either shift in resistance or sensitivity.

## Declarations

### Ethics approval and consent to participate

The protocol of this study was approved by the National Ethics Committee for Research in Human Health (N° 2020/18/082/CE/CNERSH/SP). As this was a retrospective study, the confidentiality of the information obtained on the study subjects was respected.

### Availability of data and materials

The data will be available upon reasonable request to the corresponding author.

### Competing interests

Authors' have none to declare.

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This study did not receive any funding in any form.

### Author's contributions

All authors contributed to the design and execution of the study, participated in article drafting and critical revision, and read and approved the final version of the manuscript.

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