Original Research Article

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 germs isolated based on various characteristics (morphological, cultural, and biochemical using the galleries and VITEK). The VITEK was used to perform the antibiogram on Mueller-Hinton agar (MH) for non-demanding bacteria, confirmed by the minimum inhibitory concentration (MIC) measurement. 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 *Escherichia 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

Study design

This was a retrospective study with a descriptive aim. Data for this study were obtained from patients who came for consultation at Laquintinie Hospital in Douala.

Laboratory analyses

Sample Collection and Bacterial Isolation

Identification of isolated germs was carried out according to morphological, cultural, and biochemical characteristics using API20E®, API VITEK® cards, and the coagulase test.

Antimicrobial Susceptibility Testing

The Antibiotic susceptibility pattern of the isolated E. coli strains was determined using the VITEK® automated equipment (bioMérieux, Marcy l'Etoile, France) and the solid-state diffusion method on Mueller-Hinton agar (MH) for non-demanding bacteria, and on MH with 5% sheep blood (MH-S). Resistance was confirmed by measuring the MIC.

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
Puncture fluid	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 *Escherichia 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	
Puncture Liquid	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 *Escherichia 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%).

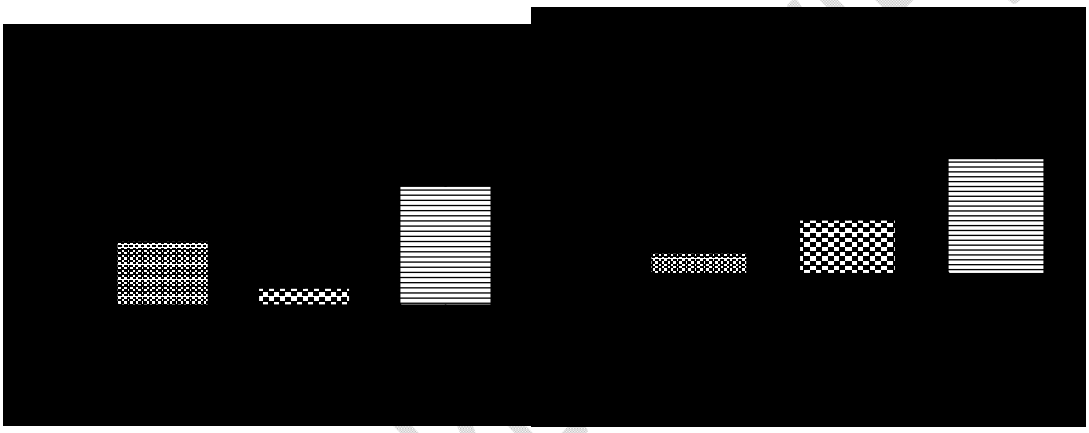

Figure 1: Distribution of the behavior of different antibiotics (Betalactamins) towards *Escherichia Coli*

Description: A: Sensible; B: Resistances; Significant < 0,005 (Kruskal-Wallis and Friedman statistic via ANOVA Analysis); **Rp**: Resistant to 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.

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* towards penicillins

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 resistance

Several carbapenem antibiotics were tested on *Escherichia Coli* during this period. Almost 1% resistance was observed with imipenem and less than 1% with Metropenem. 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

Antimicrobial	Susceptibility Test Result of 2332 Strains (n/%)		
	R	**I**	**S**
ATM	0 (0.0)	8 (1.9)	12 (2.9)
EMAIL	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**: Metropenem.

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)
X	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:** resistant category; **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**
		Coefficient de corrélation	
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 coef	
Cephalosporins		-0.14	-0.50
Carbapenems	-0.14		-0.07
Pénicillins	-0.50	-0.07	
		P value	
Cephalosporins		0.73	0.19
Carbapenems	0.73		0.86
Pénicillins	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 was 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 National Ethics Committee for Human Health Research (n° 2020/18/082/CE/CNERSH/SP) approved the experimental protocols and methods used in this study. The study was performed for the

Revised Helsinki Declaration (1989). All methods were carried out by relevant guidelines and regulations. Informed consent was obtained from all individual participants included in the study. The objectives of the study were explained to each participant and their parents/guardians, and written informed consent was obtained prior to their inclusion in the study.

Availability of data and materials

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

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