Revista Médica del
Hospital General de México

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ORIGINAL ARTICLE

# *Escherichia coli* producing extended-spectrum β-lactamases in a hospital in Mayabeque, 2022-2023

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## Abstract

**Introduction:** *The indiscriminate use of antibiotics brings with it an increase in antimicrobial resistance, especially in Gram-negative microorganisms and fundamentally in Escherichia coli, which uses the production of β-lactamases as the main mechanism of multidrug resistance.* **Objective:** *The objective of the study is to characterize the behavior of E. coli strains producing extended-spectrum β-lactamases processed in the Microbiology Laboratory of the Aleida Fernández Chardiet Hospital from January 2022 to June 2023.* **Material and methods:** *An observational, descriptive, cross-sectional study was carried out in the Microbiology Laboratory of the Aleida Fernández Chardiet Hospital between January 2022 and June 2023. The population was made up of 30 isolates of E. coli that produce extended-spectrum β-lactamases. The variables were antimicrobial resistance profile, number of samples processed, service of origin of the samples, and type of samples processed. A database was generated in Microsoft Excel 2016 for data analysis using descriptive statistics.* **Results:** *Only 30 samples (9.3%) were E. coli producers of extended-spectrum β-lactamases, 70% (21 strains) coming from outpatients, and 80% from urine samples. Amikacin was effective in 23 isolates, unlike cefepime, which showed resistance in 25.* **Conclusion:** *The frequency of isolation of E. coli producing extended-spectrum β-lactamases was low, and the highest source of positivity was obtained in urine samples and from outpatients. The most effective antimicrobials turned out to be amikacin and cefoxitin.*

***Keywords:*** *β-lactamases. Drug resistance. Microbial. Enterobacteria. Escherichia coli.*

## Introduction

At the beginning of the 20th century, the development of the antibiotic era began, it is characterized by molecules that attack bacteria, from this idea, Paul Ehrlich conceived the magic bullet project, which consists of attacking the germ and not human cells. However, it was not until 1928, when Alexander Fleming, through a stroke of luck, discovered an antibiotic that saved the lives of millions of people around the world: penicillin, for these reasons, Fleming was the initiator of the antibiotic era, characterized by the emergence of new antimicrobials with broad-action spectrums for a number of bacteria[1].

A few years later, the first bacterial strains resistant to penicillin and other antibiotics began to emerge. The ability of microorganisms to resist antibiotics and

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Date of reception: 26-10-2024
Date of acceptance: 13-12-2024
DOI: 10.24875/HGMX.24000077
Available online: 01-04-2025
Rev Med Hosp Gen Mex. 2025;88(3):128-133
www.hospitalgeneral.mx

therapy is one of the phenomena that attracts the attention of modern science and the World Health Organization (WHO). Scientists such as Vega, who belongs to the field of Microbiology, state that antibiotics begin to lose their activity against bacteria as they are addressed on a massive scale[2]. This use is given by the inappropriate use of antibiotics that bring disadvantages numerous problems and damages, among them, the increase of resistant strains around the world with more than alarming figures; for these reasons, the WHO recommends the monitoring of multidrug-resistant microorganisms, among which *Escherichia coli*, due to the production of extended-spectrum β-lactamases (ESBLs), characterized as their most notable resistance mechanism[3-5].

Bacterial strains that present ESBL enzymes are capable of hydrolyzing the β-lactam ring present in drugs such as penicillin, cephalosporin, and monobactam. These bacteria are pathogens of great importance, both in community-acquired infections and in nosocomial infections, where it is confirmed that 35.3% of bacteremias are caused by an ESBL strain[6].

Research on the spread of ESBLs began in the early 1990s, in the *Enterobacteriaceae* family and more in *E. coli*. According to Carcausto-Huamani[3] more recent studies carried out at the "Cayetano Herenia" Hospital in Peru, a prevalence of *ESBL E. coli* is identified as 28.6%, while 10 years ago, the prevalence is < 5% in the community.

The study for monitoring antimicrobial resistance trends[7] carried out in Spain reveals an increase in the frequency of *E. coli* ESBL, of community origin, in the period from 2002 to 2015, a similar situation in the United States, in its hospitals in the southeast where this type of microorganism is most frequently isolated. In Cuba, despite the low rate of studies on the subject, it is known that between 2014 and 2017, the prevalence of ESBLs-producing *Enterobacteriaceae* varies between 32 and 57%, according to different studies[7].

In the Microbiology Laboratory, of the Aleida Fernández Chardiet Teaching-Clinical-Surgical Hospital, there are isolates of *E. coli* ESBL in the processed samples, great difficulties are generated in the establishment of antimicrobial treatments, due to these reasons and the scarcity of studies on the subject in Cuba, the following research aims to characterize the behavior of the strains of *E. coli*, producers of extended-spectrum β-lactamases processed in the Microbiology Laboratory of the Aleida Fernández Chardiet Teaching-Clinical-Surgical Hospital in the period from January 2022 to June 2023.

## Material and methods

An observational, descriptive, cross-sectional study was carried out in the Microbiology Laboratory of the Aleida Fernández Chardiet Teaching-Clinical-Surgical Hospital, between January 2022 and June 2023. The study population consisted of 488 *E. coli*-positive isolates from different cultures and hospital services. The sample consisted of the 30 *E. coli* ESBL strains isolated.

The scarcity of positive cases for this bacterium is due to limitations inherent in the laboratory where the study was conducted. Its infrastructure is insufficient to process a large volume of samples and apply advanced microbiological techniques. Added to this is the reduced hospitalization capacity of the center and the presence of other microbiology laboratories in the municipality, which process most of the samples in the community. These factors contribute to the low number of cases detected annually.

Inclusion criteria: records of ESBL *E. coli* isolation that presented resistance to at least one of the antibiotics tested in the antimicrobial susceptibility test.

### *Exclusion criteria*

Records of isolates that did not have complete information, those that yielded positive isolates for a bacterial etiology other than *E. coli*, and contaminated samples.

The antibiotic susceptibility test was shown with the samples that were analyzed and processed by the technical staff of the hospital's Microbiology Laboratory, and the disk diffusion method described by Kirby Bauer was applied. The guidelines established by the Clinical and Laboratory Standards Institute (CLSI)[8], 2020 were used.

The following antibiotics were tested: Cefoxitin 30 μg, Aztreonam 30 μg, Ampicillin-Sulbactam 10/20 μg, Cefotaxime 30 μg, Cefepime 30 μg, Amoxicillin-Clavulanic Acid 30/10 μg, Nalidixic Acid 30 μg, Sulfaprin 50 μg, Meropenem 10 μg, Ampicillin 10 μg, Ceftazidime 10 μg, Ceftriaxone 30 μg, Cefuroxime 30 μg, and Cefazolin 30 μg. Non-β-lactam antibiotics were tested including Amikacin 30 μg, Levofloxacin 5 μg, Gentamicin 10 μg, Ciprofloxacin 5 μg, and Chloramphenicol 30 μg. All of them are from the commercial house, Liofilchem, in Italy. It was not possible to know the sensitivity to other antimicrobials because they did not exist in the laboratory where the study was carried out.

While antimicrobial susceptibility was assessed using the test described, limitations in laboratory infrastructure prevented the accurate determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (BCs) for each antimicrobial. Therefore, the presentation of susceptibility data is limited to qualitative interpretation; in addition, intermediate sensitivity was not taken into account, nor were the same antibiotics applied in all strains due to input deficit. The lack of quantitative data (CIM/CIB) prevents the graphical representation of the concentration-response curves.

For the phenotypic detection of ESBL, it was performed in all isolates that showed resistance to at least one of the first cephalosporins (Cefazoline 30 µg, second Cefuroxime 30 µg, third Cefotaxime 30 µg/Ceftazidime 10 µg, Ceftriaxone 30 µg, Cefoxitin 30 µg, and fourth-generation Cefepime 30 µg and with the monobactam Aztreonam 30 µg, the disc test combined with inhibitor was used: Cefotaxime 30 µg - Clavulanic acid 10 µg) in accordance with the recommendations of CLSI[8], 2020. It was incubated at 37 °C for 24 h to take the reading. Improvement in cephalosporin inhibition halo when using the disc with an inhibitor indicated ESBL production. The diameters of the zones of complete inhibition were measured in millimeters with a caliper.

The variables analyzed were resistance profile and susceptibility of antimicrobials (amikacin, cefoxitin, aztreonam, ampicillin-sulbactam, cefotaxime, cefepime, chloramphenicol, ciprofloxacin, amoxicillin-clavulanic acid, gentamicin, nalidixic acid, sulfaprim, meropenem, ampicillin, ceftazidime, ceftriaxone, cefuroxime, cefazolin, levofloxacin), number of samples processed for *E. coli* ESBL (positive and negative), Service of origin of the samples: Outpatient, Nephrology, Orthopedics, Surgery, Intensive Care and Emergency Unit and Internal Medicine, type of samples processed: urine, blood, catheter tip, secretion and surgical wound. All study variables were qualitative.

A database was generated in Microsoft Excel 2016, for the statistical analysis of the data, to which the absolute and relative frequency was determined. In addition, the percentage difference was established for the resistance variable.

The percentage difference was calculated as the difference between the value of the last year and the first, divided by the value of the 1st year and multiplied by one hundred.

**Table 1.** Susceptibility and resistance profile in *Escherichia coli* ESBL isolates to tested antibiotics

| Antibiotics | Sensible | | Resistant | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Amikacin | 23 | 76,7 | 6 | 20 |
| Ampicillin | 0 | 0 | 4 | 13,3 |
| Ampicillin-Sulbactam | 10 | 33,3 | 3 | 10 |
| Aztreonam | 9 | 3 | 20 | 66,7 |
| Amoxicillin-Clavulanic acid | 2 | 6,7 | 15 | 50 |
| Cloranfenicol | 3 | 10 | 0 | 0 |
| Ceftazidime | 0 | 0 | 18 | 60 |
| Ciprofloxacin | 3 | 10 | 14 | 46,7 |
| Gentamicin | 2 | 6,7 | 10 | 33,3 |
| Ceftriaxone | 0 | 0 | 23 | 76,7 |
| Cefotaxime | 5 | 16,7 | 19 | 63,3 |
| Cefuroxime | 0 | 0 | 13 | 43,3 |
| Cefepime | 4 | 13,3 | 25 | 83,3 |
| Cefoxitin | 19 | 63,3 | 0 | 0 |
| Cefazolin | 0 | 0 | 14 | 46,7 |
| Levofloxacin | 0 | 0 | 3 | 10 |
| Meropenem | 1 | 3,3 | 0 | 0 |
| Nalidyxic acid | 2 | 6,7 | 11 | 36,7 |
| Sulfaprin | 2 | 6,7 | 11 | 36,7 |

ESBL: extended-spectrum β-lactamases.
*Source: Microbiology laboratory sample log book.*

## Results

The susceptibility profile of the isolated ESBL *E. coli* strains was shown, the antimicrobials that presented the greatest efficacy were Amikacin and Cefoxitin with 76.7% and 63.3%, respectively, on the other hand, drugs such as Cefepime with 83.3% and Ceftriaxone with 76.7% showed greater resistance to the pathogen (Table 1).

The ambulatory health area obtained the highest positivity with bacterial growth of 70%, and the Nephrology and Internal Medicine services contributed four 13.3% and two 6.7%, respectively (Table 2).

The most common type of sample was urine, followed by peripheral blood and culture of catheter tips in liquid medium (Fig. 1).

**Table 2.** Prevalence of *ESBL-carrying Escherichia coli*

| Health services | *Escherichia coli* BLEE | |
|---|---|---|
| | No. | % |
| Ambulant | 21 | 70 |
| Nephrology | 4 | 13,3 |
| Orthopedics | 1 | 3,3 |
| General surgery | 1 | 3,3 |
| Internal medicine | 2 | 6,7 |
| ICEU* | 1 | 3,3 |
| Total | 30 | 100 |

*Intensive care and emergency unit.
ESBL: extended-spectrum β-lactamases.
*Source: Microbiology laboratory sample log book.*

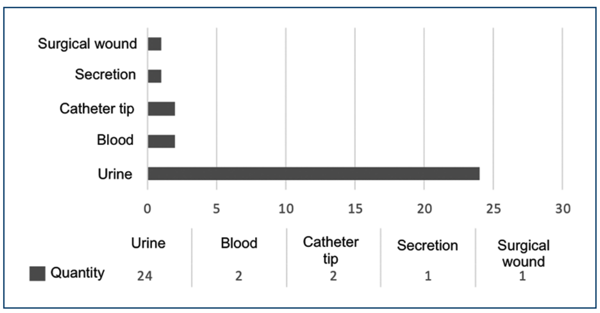


**Figure 1.** Prevalence of *Escherichia coli* extended-spectrum β-lactamases, by sample type.
*Source: Microbiology laboratory sample log book.*

## Discussion

Gram-negative pathogens and those belonging to the *Enterobacteriaceae* family such as *E. coli* are the ones that most frequently use the production of ESBL enzymes, as the main mechanism of antimicrobial resistance, favored to a large extent by the indiscriminate use of antibiotics both in the community and at the hospital level.

In a study[9] of the health sector of Huesca, it was found that the antibiotics with the best efficacy against the strains of *E. coli* are fosfomycin and nitrofurantoin, followed by gentamicin. The data obtained by Mendieta-Astudillo et al.[10] in Ecuador coincide with the previous study in its entirety.

In this research, the information does not correspond to those of the world literature due to two important aspects. The first corresponds to the lack of blotting paper discs impregnated by antibiotics such as fosfomycin and nitrofurantoin in the center, which makes it impossible to make comparisons with other studies. The second aspect is due to the displacement of Gentamicin as one of the drugs with the highest sensitivity *in vitro* against the strains of *E. coli* ESBL, isolated in the institution.

Regarding the resistance of the *E. coli* ESBL strains, in another study, Chero-Vargas et al.[11] at the Jesús del Norte Clinic in Peru, ampicillin, Ceftriaxone, and Ceftazidime are identified as the antibiotics with the highest degree of resistance, data that correspond to those found by the present research. Barroso-González et al.[12] in Havana discover a pattern of resistance quite similar to what has been proposed in previous research.

In the Microbiology Laboratory, where this research is carried out, the frequency of isolation of ESBL *E. coli* is low. In another study[13] at the National Institute of Health in Lima, Peru, 34.8% isolation was obtained, in another study from that Country, a similar behavior was achieved at 31.1%. At the Salvador Allende Hospital in Havana, the incidence of isolation of *E. coli* ESBL strains is 46%[7]; higher than that reported in the present study.

The variability in the incidence of isolation of this pathogen can be largely related to the number of samples processed by the different health centers, in the year and the time frame used by each researcher to carry out the studies.

In the III-1 hospital located in Chiclayo, Peru,[14] it was found that the incidence of *E. coli* ESBL is higher in the Internal Medicine Services with 39.2%, General Surgery 42.9%, and Emergency 41.70% in isolations that are performed on blood, secretion, and urine samples. Pinguil-Yugsi et al.[15] in Ecuador observe a higher prevalence of this pathogen in Surgery Services and in clinical areas, so its data coincide with those of the previous study. In this research, the highest number of isolations of *E. coli* ESBL corresponds to the outpatient area and the Health Service with the highest incidence is the Nephrology Department.

It is common for the Nephrology Service to be one of the most affected by these multidrug-resistant bacterial strains because most of the patients who come to this area are undergoing hemodialysis, so they have a high risk of presenting infections by resistant microorganisms due to secondary immunodeficiency due to various factors. In a study[16] carried out at the Institute of Nephrology of Havana, an isolation of *E. coli* ESBL was obtained at 68.1%, data that support the results.

The community finding of *E. coli* ESBL is increasing, this is evidenced in this study where the frequency of isolation in this area is the highest, data that correspond to those of Velazquez et al.[17] in Marcos-Carbajal et al.[18] in Peru, with 52 and 63.6% of strains found, this bacterium produces a wide range of infections in the community which allows a wide distribution and facilitates processes of recombination and transfer with a wide variety of RAM patterns.

As *E. coli* is the etiological agent par excellence of urinary tract infections, the largest number of isolates of this pathogen in the center under study are obtained from urine samples. Surgical site infections are among the main types of infection associated with extraintestinal *E. coli*, as shown by research[19], in Cuba. The data from this study do not coincide with those presented above, but they do correspond to those of Matta-Chuquisapon et al.[20], where the most frequent samples where isolation was performed were urine and blood, with 54 and 42%, respectively.

The main limitations of the study are that it is not possible to compare it with other studies in the hospital due to the lack of these. In the studies analyzed, the same antibiotics are not tested, so in some of them, it is impossible to contrast the information. In addition, the laboratory is limited to processing a small number of samples, due to a lack of resources.

## Conclusion

The frequency of isolation of *E. coli*, producers of extended-spectrum β-lactamases, is low, and the highest source of positivity is obtained in urine samples and from outpatients. The most effective antimicrobials are Amikacin and Cefoxitin. Ceftriaxone and Cefepime showed the highest resistance indices.

## Funding

The authors declare that they have not received funding.

## Conflicts of interest

The authors declare no conflicts of interest.

## Ethical considerations

**Protection of humans and animals.** The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki Addendum of Taiwan. The procedures were approved by the institutional Ethics Committee.

**Confidentiality, informed consent, and ethical approval.** The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary. Relevant guidelines were followed. Privacy was ensured through the encryption of patient data, which were obtained in the sample record book of the Microbiology Laboratory, and were treated with strict confidentiality, avoiding violating their rights. The information was used for investigative purposes only, was not released to the public, and no new investigations were conducted.

**Declaration on the use of artificial intelligence.** The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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