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Resistance to Antibiotics in Clinical Isolates of Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* Obtained from Urine Cultures in Patients with Urinary Tract Infection

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Abstract

The objective of this study was to identify strains of *Escherichia coli* producing extended spectrum beta-lactamases (ESBL) in urine cultures and to evaluate the patterns of resistance to certain antibiotics used in clinical practice as treatment of choice in the Valley of Toluca, Mexico. One hundred and fifty-five urine samples were obtained from people in the Toluca Valley, Mexico, under the necessary hygiene conditions, 100 ml used to identify the pathogen and its resistance patterns were collected from each sample through an automated WalkAway method. In order to determine ESBL, a double disk technique was used. Nineteen ESLE-producing strains of *E. coli* were obtained, of which 100% showed resistance to ampicillin and cephalosporins, to tetracycline 93.75% resistance, levofloxacin 87.5% and ciprofloxacin 81.25%, and sensitivity of 100% to amikacin and carbapenemics. In conclusion, antibiotic resistance represents a major health problem. As the present study shows, ampicillin is a drug of choice for urinary tract infectioncaused by *E. coli,* but in the Toluca Valley the resistance to this antibiotic is 100% in ESBL-producing strains, which also show high resistance patterns to cephalosporins, quinolones, tetracycline and trimethoprim with sulfamethoxazole.

Keywords: sensitiviy, antibiotic, resistance, infection, *E coli*

Резюме

Целта на настоящото проучване е да се идентифицират щамовете на *Escherichia coli*, продуциращи беталактамази с удължен спектър (ESBL) в уринарните култури, както и да се оценят моделите на резистентност към определени антибиотици, използвани в клиничната практика като лечение по избор в долината на Толука, Мексико. Събрани са 155 проби от урина от хора в долината Толука, Мексико при необходими хигиенни условия. За идентифициране на патогена и неговите модели на резистентност са използвани 100 мл. от всяка проба, използвайки автоматизиран метод на Walkaway. За да се определи ESBL, беше използвана двойна дискова техника. Получени са 19 щама, произвеждащи ESLE на *E. coli* и 100% от тях показват резистентност към ампицилин и цефалоспорини, 93.75% - към тетрациклин, 87.5% - към левофлоксацин и 81.25% - към ципрофлоксацин. Едновременно с това е отчетена 100% чувствителност към амикацин и карбапенемици. В заключение, антибиотичната резистентност представлява основен здравословен проблем. Както показва настоящото проучване, ампицилинът е лекарство за избор при инфекция на пикочните пътища, предизвикана от *E. coli*, но в долината на Толука резистентността към този антибиотик е 100 в щамовете, произвеждащи ESBL. Установени са също модели с висока устойчивост към цефалоспорини, хинолони, тетрациклин и триметоприм със сулфаметоксазол.

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Introduction

According to the World Health Organization (WHO), urinary tract infections (UTIs) are the most common infections in humans (WHO, 2017). Globally, approximately 150 million people are diagnosed with UTIevery year (Tuem *et al*., 2019). They occur as a result of bacterial proliferation in the urinary system, manifesting itself as symptomatic or asymptomatic bacteriuria (Zhao *et al*., 2020).

The process by which the urinary tract maintains the sterility of the urine is the continuous unobstructed flow (Soman and Yuxuan, 2015). That is why, abnormalities in the anatomy or function that hinder that flow compromise urine sterility, giving way to infections (Hobeman *et al*., 2003). Most urinary tract infections begin with the invasion of bacteria from the urinary tract via the ascending route (Baldassarre and Kaye, 1991), generally due to bacteria originating from the digestive tract (Beyene and Tsegage, 2011; Tuem *et al*., 2019). These infections may be limited to the lower tract or bladder (acute cystitis), or may involve the kidney as a kidney or upper tract infection (acute pyelonephritis) (Nicolle, 2002).

The epidemiology of infection varies with sex, age, and the presence of associated genitourinary abnormalities (Nicolle, 2002). UTIs can occur in male patients, but, more frequently in female patients (Willy *et al*., 2015), attributed to the proximity of the genital tract and shorter urethra. Specific subpopulations at increased risk of urinary tract infection include infants, pregnant women, the elderly, patients with spinal cord injury and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome, human immunodeficiency virus, and patients with underlying urological abnormalities (Nicolle, 2019).

Urinary tract infections (UTI), both acquired in hospital and in the community, (Sweileh *et al*., 2018), have quite a detrimental influence on the patient‘s quality of life. The most frequently isolated microorganisms are mainly *Escherichia coli,* followed by *Staphylococcus saprophyticus, Citrobacter spp, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, P. mirabilis, Klebsiella spp, S. aureus, Salmonella spp., Enterococcus spp*., and *Enterococcus spp*. (Tuem *et al*., 2019).

Antimicrobial therapy is essential for the treatment of urinary tract infections to prevent complications (Hooton and Stamm, 1997). Today, the emergence of antimicrobial resistance represents a serious threat to public health worldwide (Li and Webster, 2018). Uropathogens belonging the *Enterobacteriaceae* family are known to produce extensive-spectrum beta-lactamases (ESBLs) and carbapenemases, leading to resistance to multiple drugs (Perez and Van-Duin, 2013). In particular this applies to *E. coli* (Gajamor *et al*., 2018). This uropathogen is the main causal agent isolated prevalently in uncomplicated UTI in Mexico. Its resistance to antimicrobials translates into a substantial financial burden on the health system, as well as significant morbidity for patients (Reyes and Ladón, 2016; Lagunas-Rangel, 2018).

Materials and Methods

In the present study, 155 urine cultures were performed in patients with clinical symptoms of urinary tract infection, without exclusion criteria such as gender or age, in a town in the center of the Toluca Valley, Mexico. Of the 155 urine cultures performed, the distribution was: 107 people of the female gender representing 69% of the study population, and 48 men representing 31%. The age rangelower limit was 1-year-old patient, and the highest limit was a 91-year-old patient, with an average age of 25 to 30 years.

For the study, 100 ml of the first urine of the day were collected under the necessary hygiene conditions for the collection of the specimen. The identification of the etiological agent as well as the sensitivity tests were performed using the automated method WalkAway SI 96 from Beckman Coulter. For the detection of extended-spectrum beta-lactamases, the double disc method was used.

Results

Among the 155 urine cultures performed, 80 were positive for microbial development, and 75 showed no development.

The 51.62% that represents positive cases have a distribution 47.5% represented bacteria other than *E coli*, 8.75% corresponded to yeasts and 43.75% represented *E. coli*, which is equivalent to 35 cultures. Of the 35 isolated from *E. coli*. 16 strains show production of extended spectrum beta-lactamases (ESBL) equivalent to 45.72%. Table 1 shows the resistance patterns to the different antibiotics used, in the clinical isolates of *E coli* with production of ESBL.

The strains showed marked 100% resistance to second- and third-generation cephalosporins, which are broad-spectrum antibiotics, as well as to ampicillin with the same percentage, while resistance to tetracycline was found to be 93.75%.

Table 1. Antibiotic resistance of used clunic isolates

Antibiotic	Sensitivity pattern		
	Resistant	Intermediate Sensitivity	Sensitive
Amikacin	0	0	16
Amoxicillin / Clavulanic Acid	4	2	10
Ampicillin	16	0	0
Ampicillin /Sulbactam	12	1	3
Cefazolin	16	0	0
Cefepime	16	0	0
Cefotaxime	16	0	0
Ceftazidime	16	0	0
Ceftriaxone	16	0	0
Cefuroxime	16	0	0
Ciprofloxacin	13	0	3
Ertapenem	0	0	16
Gentamicin	9	1	6
Imipenem	0	0	16
Levofloxacin	14	0	2
Meropenem	0	0	16
Piperacillin/ Tazobactam	0	5	11
Tetracycline	15	0	1
Tigecycline	1	1	14
Tobramycin	9	1	6
Trimethoprim/ Sulfamethoxazole	12	0	4

Carbapenems and amikacin showed greatest sensitivity. Other antibiotics, such as quinolones, sulfa and other beta-lactams showed variable resistance (Fig. 1.).

Discussion

The extended-spectrum beta-lactamases have a broad inhibitory activity for beta-lactam drugs, such as penicillin derivatives, and first-, second-, third- and fourth-generation cephalosporins, which is demonstrated in the 16 isolates with ESBL production with a resistance pattern of 100% for amplicillin and cephalosporins.

Beta-lactams with beta-lactamase inhibitors showed variable resistance patterns, ESBLs are known not to have strong activity against these inhibitors. In the case of ampicillin with sulbactam, the resistance pattern is 75%, while for amoxicillin with clavulanic acid the resistance is only 25%. The strains show a resistance pattern of 0%, but an intermittent sensitivity of 31.25% to piperacillin with tazobactam.

Although carbapenems are considered to belong to the beta-lactam family, extended- spectrum beta-lactamases have no activity whatsoever for this class of antibiotics, so the pattern of sensitivity to meropenem, imipenem, and ertapenem is 100%.

On the other hand, a high resistance pattern is shown in other classes of antibiotics, this due to the influence of extended-spectrum beta-lactamases. In the case of quinolones, a resistance pattern of 81.25% is observed for ciprofloxacin, while for levofloxacin the resistance pattern is 87.5%. For tetracycline, there is a 93.75% resistance pattern, also affected by the presence of ESBL. The only antibiotic that showed 100% activity against ESBL-producing isolates is the aminoglycoside amikacin, and a high percentage of sensitivity was observed for tigecycline.

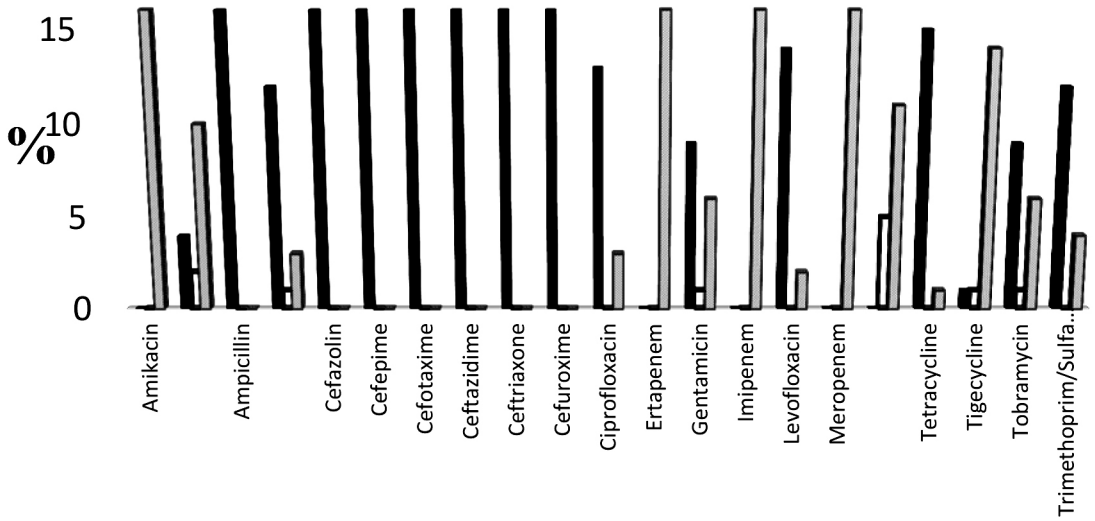

Fig. 1. Resistance and sensitivity patterns: resistant isolates - black bars; the strains with intermediate sensitivity – white bars; strains with sensitivity to the antibiotic - gray with lines

Conclusions

E. coli is one of the main known uropathogens and in many cities it represents the main causative agent of UTI. Enterobacteriaceae have acquired many mechanisms of resistance to antibiotics, and one of the main mechanisms is the production of extended-spectrum beta-lactamases, which confers resistance to beta-lactam antibiotics as well as to other classes, such as quinolones and tetracyclines.

In the present study, almost 50% of the *E. coli* isolates produced ESBL, which complicates the treatment of urinary tract infections. Therefore, first-choice therapeutic strategies face high resistance patterns.

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