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IDENTIFICATION OF *ESCHERICHIA COLI* STRAINS IN THE VAGINAL CULTURES OF HEALTHY WOMEN AND THEIR PATTERNS OF ANTIBIOTIC RESISTANCE

Jorge Angel Almeida Villegas[1,2 A,E]
• ORCID: 0000-0002-3804-9550

Harold Mondragon Reyes[2 E,F]
• ORCID: 0000-0002-8815-1510

Mariana Aguilar Sánchez[2 E,F]
• ORCID: 0000-0001-9293-3235

Maria Fernanda Cruz Rosas[2 B,C]
• ORCID: 0000-0002-6797-9263

Regina Sanchez Monroy[2 B,C]
• ORCID: 0000-0003-2621-4237

Jesus Emmanuel Bernal Zamudio[2 C,D]
• ORCID: 0000-0002-5834-9278

Ana Paula Palacios Rodriguez[3 B,C]
• ORCID: 0000-0003-2687-3208

[1] Professional School of Nutrition, Nursing and Gerontology, IUEM University, Mexico

[2] School of Biological Pharmaceutical Chemistry, University of Ixtlahuaca CUI, Ixtlahuaca, Mexico

[3] Laboratory of Environmental Microbiology and Biotechnology, National University of San Marcos, Peru

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ABSTRACT

Background: Bacterial vaginosis is the most common cause of vaginitis in women of childbearing age, and it predominantly affects young sexually active women. *Escherichia coli* is one of the most common bacteria found in the genital tract of non-pregnant (9–28%) and pregnant women (24–31%). *E. coli* strains can colonize the vaginal and endocervical regions in pregnant women, and may lead to the development of urinary tract, intra-amniotic or puerperal infections.

Aim of the study: Isolation and identification of the antibiotic resistance patterns of extended spectrum beta-lactamase (ESBL)-producing and non-producing *E. coli* in the vaginal cultures of healthy women.

Material and methods: Vaginal samples were taken from 55 healthy women. For the bacterial identification and resistance patterns, automated equipment from Beckman Coulter was used. Phenotypic techniques were used to confirm the presence or absence of ESBL.

Results: Fifty-five cultures developed *E. coli*, with the rest of the strains corresponding to different bacteria. Of the 55 *E. coli* cultures, 35 (63.63%) were ESBL-producing and 20 (36.36%) did not produce ESBL. There was an 80% resistance to penicillin, and a 76.4% and 65.5% resistance to the first and fourth generation cephalosporins, respectively. A 45.5% resistance was observed for the fluoroquinolones, 52.7% for trimethoprim/sulfamethoxazole, and 100% sensitivity to carbapenemics and amikacin.

Conclusions: A large presence of vaginal ESBL-producing *E. coli* was observed in healthy women, which increases the risk of therapeutic failure due to high levels of antibiotic resistance.

KEYWORDS: *E. coli*, antibiotic resistance, vaginal culture

Background

Vaginal infections are a frequent reason for primary healthcare consultations around the world, but their prevalence and etiology vary in different populations. The proper clinical diagnosis of these infections is necessary to establish an effective therapy, and this is fundamentally associated with recognizing the etiology [1]. Female urogenital infections are highly prevalent. Vaginal infections affect 70% of women during their lifetimes and account for millions of annual doctors' visits [2]. Vaginal ecology is influenced by factors such as the glycogen content in epithelial cells, glucose, pH, hormone levels, trauma caused by sexual intercourse, contraceptive methods, age, and antimicrobial treatments [3].

The vaginas of women of different ethnicities are inhabited by a variety of microorganisms, known as the vaginal microbiota, in varying quantities and proportions. Among these, *Lactobacillus* spp., in particular *L. crispatus, L. jensenii*, and *L. inners*, are the most prevalent bacteria in the vaginal ecosystem of healthy Caucasian women. According to the World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Lactobacilli that colonize the human vagina produce antimicrobial substances that counteract the growth of pathogenic microorganisms. Nevertheless, for reasons not completely elucidated, the vaginal microbiota composition can change, and this alteration of the ecosystem can lead to vaginal dysbiosis and infections associated with various adverse health outcomes [4]. The vaginal microbiota provides the first line of defense against sexually transmitted infections (STIs). *Lactobacillus* spp. produce lactic acid and other antimicrobial compounds that maintain a protective environment, and the absence of these bacteria is associated with an increased risk of contracting STIs. Recent large-scale molecular surveys of the vaginal microbiota have revealed five broad vaginal bacterial community-state types (CSTs). Four CSTs are dominated by *Lactobacillus* spp., while a fifth is deficient in *Lactobacillus* and comprised of a diverse set of strict and facultative anaerobes [5].

Vaginal infections represent a significant female health problem due to issues such as a high recurrence rate, drug resistance, and the emergence of persistent strains. Improvements in the therapeutic efficacy of conventional formulations intended for vaginal delivery remain as a challenge due to anatomy and physiology of the vagina, since the secretion and renewal of vaginal fluids contribute to removal of the therapeutic agent. Vaginal disorders can be categorized according to their etiologic agent into bacterial, fungal, or parasitic infections. Thus, it is important to understand the clinical and pathological features, as well as the main challenges involved in the treatments, for each type of vaginal infection [6].

Currently, vaginal infections are mainly caused by pathogenic or commensal microorganisms that, due to immunosuppression, can become pathogenic agents for humans. Bacterial vaginosis (BV), candidiasis, and trichomoniasis are responsible for the majority of vaginal infections in women of reproductive age. Abnormal vaginal discharge, itching, burning sensations, irritation and discomfort are frequent complaints among patients who visit obstetrics and gynecological clinics. However, several vaginal infections present with few or no symptoms [7].

BV is the most common cause of vaginitis in women of childbearing age. This condition predominantly affects young sexually active women but can also occur in the absence of sexual intercourse [8]. A group of microorganisms is present simultaneously in the vaginas of women with BV. The main members of this group are *Gardnerella vaginalis,* anaerobic gram-negative rods belonging to the genera *Prevotella, Porphyromonas*, and *Bacteroides*, *Peptostreptococcus* species, *Mycoplasma hominis, Ureaplasma urealyticum,* and *Mobiluncus* species. In addition, facultative species of *Lactobacillus* tend to be present in lower concentrations compared to women with a normal vaginal exam [9].

Escherichia coli is reported as one of the most common organisms found in the genital tracts of non-pregnant (9–28%) and pregnant women (24–31%). Vaginal *E. coli* can colonize the vaginal and/or endocervical regions in pregnant women, and can lead to the development of urinary tract, intra-amniotic and puerperal infections through 'fecal-vaginal-urinary/neonatal' transmission [10].

Aim of the Study

The aim of this study is to isolate and identify antibiotic resistance patterns in extended spectrum beta-lactamase (ESBL)-producing and non-producing *E. coli* in the vaginal cultures of healthy women.

Material and methods

Study population and data collection

The study group was comprised of 55 patients that attended an outpatient clinic in the Toluca Valley in Mexico. Vaginal cultures were taken from healthy women, whose only exclusion criterion was presenting with a sign or symptom of vaginal infection in the week or days prior. The age of the participants was not an exclusion criterion as one of the objectives was to identify the age at which the highest prevalence of

bacterial growth is discovered and whether there is a presence of ESBL-producing *E. coli*.

To take the culture, a swab was made and was placed in Stuart transport medium for 5 hours after sampling. Sheep blood agar and chromogenic agar were used for the cultures, which were incubated for 24 hours at 37 °C. The petri dish with chromogenic agar was used for a reseeding with standard medium, and an OD of 0.5 on the MacFarland scale was used to perform bacterial identification in a panel for gram negative bacteria, as well as for the susceptibility tests. This was done using Beckman Coulter equipment. For the determination of minimum inhibitory concentrations (MIC) in the antibiogram, the concentrations established by the Clinical and Laboratory Standards Institute (CLSI) were used. The confirmation of ESBL was made using a disk synergy test with ceftazidime and clavulanic acid [11].

Variables

Age, presence of ESBL, and resistance to fluoroquinolones.

Statistical analyses

The statistical analyses were performed using IBM SPSS v. 22 software. To establish the relationship between the variables "Bacteria ESBL" and "Age", Chi squared tests and point-biserial correlations were used. The null hypothesis (Ho) was "the presence of ESBL bacteria is not related to the age of the women" and the alternate hypothesis (Ha) was "the presence of ESBL bacteria is related to the age of the women." The 55 patients from whom vaginal culture samples were collected were subjected to hypothesis testing. In addition, the phi coefficient was used to establish the correlation between the variables "Bacteria *E. coli* ESBL" and "resistance to fluoroquinolones".

Results

One hundred and ten cultures were obtained from healthy women of different ages, of which only 100 cultures showed microbial growth. Fifty-five cultures developed *E. coli* and the rest of the strains corresponded to different bacteria. Of the 55 cultures of *E. coli*, 35 63.63% (35) were strains that produced ESBL, and 20 36.36% (20) were strains that did not produce ESBL but may have had the presence of AmpC beta-lactamases.

The average age of the participants was 33.94 years, an middle of 29, with the lowest age being 17 years and the highest 70 years. All samples were obtained from healthy women with an absence of vaginal, cervicovaginal or urinary tract infections.

The patterns of resistance to the different antibiotics are shown in Table 1, where the number of sensitive and resistant *E. coli* cultures are listed.

Table 1. Antibiotic resistance patterns in *E. coli* isolates (n=55)

Antibiotic	***E. coli* Resistant (n/%)**	*E. coli* **Sensitive (n/%)**
Ampicillin	44/80	11/20
Ampicillin/Sulbactam	27/49.1	28/50.9
Amoxicillin/Clavulanic acid	21/38.2	34/61.8
Cefalothin	42/76.4	13/23.6
Cefepime	36/65.5	19/34.5
Ceftriaxone	40/72.7	15/27.3
Ceftazidime	40/72.7	15/27.3
Cefotaxime	40/72.7	15/27.3
Cefuroxime	39/70.9	16/29.1
Piperacillin/Tazobactam	6/10.9	49/89.1
Imipenem	0/0	55/100
Meropenem	0/0	55/100
Ertapenem	0/0	55/100
Tigecycline	12/21.8	43/78.2
Tetracycline	29/52.7	26/47.3
Amikacin	0/0	55/100
Gentamicin	16/29.1	39/79.9
Levofloxacin	25/45.5	30/54.5
Ciprofloxacin	25/45.5	30/54.5
Trimetroprim/Sulfametoxazol	29/52.7	26/47.3

For penicillin derivatives, 80% resistance was observed, which is to be expected due to the presence of 63.3% of ESBL-producing strains. However, the presence of other beta-lactamases such as AmpC is also reflected in the number of strains resistant to this antibiotic. Cephalosporins from the first to fourth generations had percentages of 76.4% (42) and 65.5% (36) for cefalothin and cefepime, respectively. Ampicillin/sulbactam had a resistance of only 49.1% (27), while amoxicillin/clavulanic acid exhibited a resistance of 38.2% (21), a lower percentage than ampicillin alone.

Carbapenemics meropenem, imipenem and ertapenem had a sensitivity of 100% for all drugs, which indicates an absence of carbapenemase production. In the case of the aminoglycoside amikacin, it also had a sensitivity of 100%, but gentamicin from the same family had a 29.1% (16) resistance.

Fluroquinolones, ciprofloxacin and levofloxacin had a resistance of 45.5% (25) for both drugs, which represents a high rate. For tetracycline and trimethoprim with sulfamethoxazole, there was a resistance of 52.7% (29). High resistance rates were also observed for other drugs such as tigecycline with 21.8% (12), and beta-lactams with beta-lactamase inhibitors such as piperacillin/tazobactam with 10.9% (6).

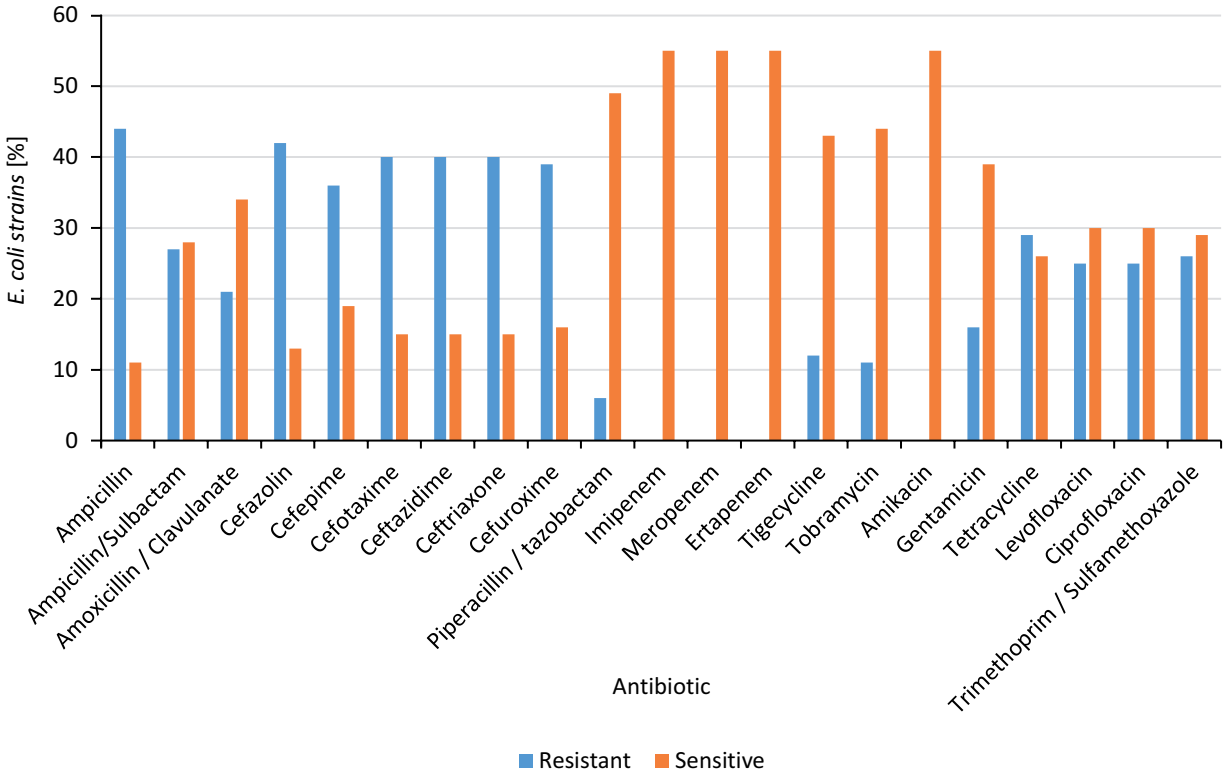

Figure 1. Antibiotic resistance patterns in *E. coli* isolates

Figure 1 shows these resistance trends and the comparisons between those strains sensitive and resistant to the drugs evaluated in the study.

ESBL-producing bacteria and Age

A Chi-squared test indicated that there was no significant association between the presence of ESBL-producing bacteria and the age of the patients (χ^2=29.648, p=0.484).

The point-biserial correlation (r=-0.055, p=0.05) indicated an inverse relationship between the variables, which means the older the patients, the lower the probability of the presence of ESBL-producing bacteria.

ESBL-producing bacteria and fluoroquinolone resistance

The phi coefficient (ϕ=0.311, p=0.021) indicated a significant association between ESBL-producing bacteria and resistance to fluoroquinolones.

Discussion

Key results

A high level of ESBL was detected by the biochemical tests, this was reflected in the high index of resistance (80%) for ampicillin, which is hydrolyzed by these enzymes. The high resistance rate also indicates a high number of AmpC-producing strains, as there were only 35 ESBL-producing strains. In the case of first-generation cephalosporins, such as cephalothin, there was a resistance seen in 42 strains (76.4%), which also implies the existence of AmpC-producing strains. A similar pattern of results was observed for cefuroxime with 39 resistant strains (70.9%), and 40 strains were resistant to third generation cephalosporins (72.7%).

Interpretation

Both AmpC and ESBL do not have the ability to hydrolyze beta-lactamase inhibitors, so there was little resistance to amoxicillin/clavulanate (61.8%, 34), ampicillin/sulbactam 50.9% (28), and piperacillin/tazobactam 89.1%, (49) [12,13]. On the other hand, a high percentage of resistance to tetracyclines was observed. Tetracycline and tigecycline showed resistance rates of 52.7% (29) and 21.8% (12), respectively. Bacterial resistance to tetracycline is usually mediated by energy-dependent pumping of tetracycline out of the bacterial cell. The genes tet (A), (B), (C), (D), (E), (Y) and (I) in gram-negative bacteria encode these efflux systems [14].

In the case of quinolones, a resistance pattern of 45.5% (25) was observed for both levofloxacin and ciprofloxacin. In *E. coli*, resistance to quinolones frequently occurs through mutations in the gyrA gene

and, less often, in the gyrB gene, which catalyze ATP dependent DNA supercoiling. Other mechanisms of *E. coli* resistance to quinolones and fluoroquinolones are through efflux pumps and reduced drug accumulation in the bacteria due to changes in the purine protein [15].

Conclusions

There is a high presence of vaginal *E. coli* strains that produce ESBL and AmpC-type beta-lactamases in apparently healthy women. In addition, a high degree of resistance to antibiotics such as quinolones, tetracyclines and trimethoprim with sulfamethoxazole was observed for these strains. These families of antibiotics, along with the beta-lactam drugs, are first line therapeutics for infections of this nature, which will lead to therapeutic failure. The relationship between the presence of ESBL and resistance to quinolones also indicates a risk of failure in therapy with these antibiotics.

Code of ethics

The ethics committee of Pasteur Laboratories approved this study. This research did not compromise the security and identity of the participants as samples collected are considered routine.

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Correspondence address:
Jorge Angel Almeida Villegas
Professional School of Nutrition,
Nursing and Gerontology,
IUEM University, Mexico
E-mail: angel.almeida@uicui.edu.mx

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