



Caracterización de infección del tracto urinario a partir de urocultivos positivos aislados de mujeres embarazadas de la ciudad de Machala año 2022

Characterization of urinary tract infection from positive urine cultures isolated from pregnant women in the city of Machala year 2022

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Resumen

Introducción. Las infecciones del tracto urinario (ITU) durante el embarazo incrementan el riesgo de morbimortalidad materna y neonatal. Las bacterias, debido a su gran capacidad de acondicionamiento, logran desarrollar varios mecanismos de resistencia a antimicrobianos que anteriormente eran usados para eliminarlos; siendo las enterobacterias BLEE las de mayor prevalencia a nivel global. **Objetivo.** Caracterizar las principales enterobacterias causantes de ITU, en mujeres embarazadas atendidas en centros de salud de la ciudad de Machala. **Metodología.** Esta investigación tuvo un enfoque cuantitativo, descriptivo documental secundario, de corte transversal realizado a partir de los registros de urocultivos positivos del laboratorio de microbiología del Hospital General IESS Machala y Laboratorio Clínico Solidario. **Resultados.** Se evidenció que el 85.5% (83/97) de urocultivos positivos causantes de ITU pertenecen a Enterobacterias; siendo aquellas especies uropatógenas productoras de BLEE con mayor dominancia en este estudio la *E. coli* (91.6%) y *Klebsiella aerogenes* (1.2%). **Conclusión.** *Escherichia coli* y *Klebsiella aerógenes* fueron las enterobacterias productoras de BLEE con mayor resistencia a antibióticos como FEP, CRO y KF (cada una con un 50% de resistencia), pudiendo estar relacionado con el bajo costo de dichos medicamentos; por ello es importante proporcionar una educación continua en salud y revisiones constantes acerca del perfil de resistencia antimicrobiana para disminuir las repercusiones de las ITU y de las bacterias con mayor resistencia en mujeres embarazadas.

Abstract

Introduction. Urinary tract infections (UTI) during pregnancy increase the risk of maternal and neonatal morbidity and mortality. Bacteria, due to their great conditioning capacity, manage to develop several resistance mechanisms to antimicrobials that were previously used to eliminate them; Enterobacteriaceae ESBL being the most prevalent globally. **objective.** To characterize the main enterobacteria causing UTI in pregnant women treated in health centers in the city of Machala. **Methodology.** This research had a quantitative, descriptive, secondary documentary, cross-sectional approach

carried out from the records of positive urine cultures from the microbiology laboratory of the IESS Machala General Hospital and Solidarity Clinical Laboratory. Results. It was shown that 85.5% (83/97) of positive urine cultures causing UTI belong to Enterobacteriaceae; the ESBL producing uropathogenic species with the greatest dominance in this study were E. Coli. and Klebsiella aerogenes (1.2%). Conclusion. E. coli and Klebsiella aerogenes were the ESBL-producing enterobacteria with the greatest resistance to antibiotics such as FEP, CRO and KF (each with 50% resistance), which may be related to the low cost of said medications; Therefore, it is important to provide continuous health education and constant reviews about the antimicrobial resistance profile to reduce the repercussions of UTI and bacteria with greater resistance in pregnant women.

Introduction

Urinary tract infection (UTI) is defined as the growth of microorganisms in sterilely collected urine in a patient with compatible clinical symptoms; they may present as asymptomatic bacteriuria, acute cystitis or pyelonephritis.(1,2)They usually occur between the ages of 16 and 35, and more than 10% of women get an infection annually. The risk of UTI increases with age, with 30% to 50% of women over 50 having one, and it is believed that all women will have had a UTI during their lifetime, with 10% to 60% of these women being symptomatic.(3,4).

UTIs are considered a global health problem. Women are the most affected due to the anatomy of the urinary tract and its proximity to reproductive organs and the rectum. In this context, women are considered a vulnerable group during pregnancy and the perinatal period, which is why they constitute a group of great interest for the clinical study of urinary tract infections.(3). UTIs are common during pregnancy, which increases the risk of maternal and neonatal morbidity and mortality.(5)The most important factor that predisposes women to develop this condition during pregnancy is asymptomatic bacteriuria (ASB). If it is not treated during this stage, the development of pyelonephritis is possible, the latter being the most common cause of septic shock in pregnant women.(1).

UTIs were among the top 4 causes of death globally attributable to and associated with antimicrobial resistance due to infectious syndrome in 2019. Escherichia coli and

Klebsiella pneumoniae are the main bacteria contributing to antimicrobial resistance (AMR) in 2019, and have been identified as priority pathogens by the WHO.(6).

With reference to the Enterobacteriaceae family, a comprehensive systematic review and meta-analysis was published in 2019, in which the global prevalence of ESBL-producing Enterobacteriaceae (ESBL-P) in urinary tract infections, asymptomatic and symptomatic bacteriuria among pregnant and postpartum women was determined. Essentially, twenty-three studies (six from Africa, two from North America, one from South America, 12 from Asia and two European studies) were included, reporting data on 20,033 Enterobacteriaceae strains. The pooled prevalence of ESBL-P beta-lactamase-producing Enterobacteriaceae was 25%. The estimated prevalence rates were 45% in Africa, 33% in India, 15% in other Asian countries, 5% in Europe, 4% in South America and 3% in North America ($p < 0.001$). This estimate was 21% (95% CI 11, 31%) in patients with symptomatic UTI and was 28% (95% CI 15, 41%) in patients with asymptomatic bacteriuria ($p = 0.40$). (7).

In the national context, in the Basic Hospital of Paute, in 2018 it was published that, out of a total of 67 patients hospitalized with a diagnosis of urinary tract infection, *Escherichia coli* was characterized as the most frequent causal agent of urinary tract infection; being diagnosed in 44.8% of cases. In addition, it is included that the antibiotics for which there was greater resistance were dicloxacillin (100%), ampicillin (95.2%) and trimethoprim/sulfamethoxazole (81%), and less resistance, gentamicin (37.5%), amikacin (35.7%) and meropenem (0%).(8).

In an observational study based on 73 clinical histories of patients treated at the Carlos Andrade Marín Specialty Hospital in Quito, 71.23% of women had urinary tract infection. *Escherichia coli* was frequent in 48.39%, with greater resistance to Cotrimoxazole.(9). For its part, in the General Hospital of Ambato, in a similar study with 233 patients, *Escherichia coli* was determined by culture to be the main etiological agent in 42.49%, and the presence of ESBL-producing *Escherichia coli* in 2.58% is striking.(10).

UTIs are one of the main causes of morbidity around the world. In Ecuador, according to data from the “Global Burden of Diseases” portal, UTIs represent 0.16% of Disability Adjusted Life Years (DALYS) in the population, reaching 0.33% (9).

The diagnosis of UTI is made from the clinical history (symptoms), microscopic examination of urine (MOU) and confirmation by urine culture, this last analysis being considered the gold standard for the diagnosis of UTI; its effectiveness will depend on factors such as the appropriate collection of the urine sample.(4,10).

Based on the above, it has been considered necessary to carry out a study in the city of Machala related to the characterization of the main enterobacteria causing urinary tract

infection (*Escherichia coli* and *Klebsiella pneumoniae*), in pregnant women treated in health centers in the city of Machala, which is why through this research we intend to provide updated information and a scientific basis on the proposed topic.

Methodology

This study is of a positivist type with a quantitative, descriptive, secondary documentary approach, cross-sectional, carried out from the records of positive urine culture results from the microbiology laboratory of the IESS Machala General Hospital and the Solidarity Clinical Laboratory. The study universe was made up of pregnant patients who presented UTI, between the ages of 20 and 30, during the period January and December 2022. The sampling was non-probabilistic by total coverage. The data were processed using descriptive statistics, frequency analysis and chi square.

Inclusion criteria: registry of pregnant patients between 20 and 30 years of age who underwent urine cultures, registry of isolates that present resistance to at least one family of antimicrobials, registry of isolates that present resistance to 1st, 2nd, 3rd generation cephalosporins and monobactams, phenotypically identified as producers of extended spectrum beta-lactamases (ESBL), registry of isolates that present resistance to 4th generation cephalosporins and carbapenems phenotypically identified as producers of carbapenemases of the KPC, AMPC type.

Exclusion criteria: incomplete records of clinical isolates, record of isolated pathogenic microorganisms that are not part of the Enterobacteriaceae family.

The sample size obtained was 97 patient records based on the inclusion and exclusion criteria indicated.

Procedure

Microbiological inoculation: Bacteriological inoculation was performed on urine samples collected from the second stream, after cleaning the genital area. They were inoculated on CHROMagar Orientation plates (Becton Dickinson) with a calibrated 0.001 ml loop and incubated at 37°C in an oven for 24 hours.(11,12).

Identification and antimicrobial susceptibility: Bacterial identification was performed phenotypically using the following biochemical tests: urea, sulfide indole motility (SIM), triple sugar iron (TSI), lysine decarboxylation, citrate. In performing the antibiogram, a 0.5 McFarland scale was used with the study isolates. Antimicrobial susceptibility was performed according to the guidelines established for outpatient urinary tract infection in patients without underlying pathology by the CLSI 2020.(13).

Confirmatory ESBL test: the recommendations described by CLSI 2021 were used. The bacterial suspension was obtained and inoculated onto Mueller-Hinton agar.

Antimicrobial susceptibility discs of ceftazidime (30 µg), cefotaxime (30 ug), cefepime (30 ug), aztreonam (30 ug) (center to center) and a disc with amoxicillin/clavulanic acid were placed. Synergistic effect was interpreted as a positive result.(14).

Identification of Gram-negative bacilli and analysis of antimicrobial susceptibility using VITEK 2 compact equipment: The VITEK®2 GN identification card was used for the automated identification of Enterobacteriaceae, as well as non-Enterobacteriaceae Gram-negative bacteria and other highly pathogenic organisms. VITEK® 2 AST cards were used for antimicrobial susceptibility analysis.

Statistical processing: For statistical analysis, a database was generated in the IBM STATISTICS SPSS 22 program. The data were processed using descriptive statistics, measures of central tendency (mean, median and mode) and measures of non-central tendency (quartiles, deciles and percentiles), measures of dispersion (range, variance, standard deviation, Pearson deviation coefficient), frequency analysis and chi square.

For the presentation of the results, single and double entry tables, cross tables were used and the graphs were represented using frequency polygons and sector diagrams.

Ethical aspects:Regarding ethical aspects, personal information was kept under the ethical principles postulated in the Declaration of Helsinki Addendum of Taiwan(15,16), maintaining the confidentiality of the data obtained and without violating the rights of patients. Finally, approval from a Bioethics Committee was not required due to the research guidelines initially proposed.

Results

In the time period established in this study (January to December 2022), a total of 97 positive urine culture results were obtained in pregnant women aged 20 to 30 years. Of this total, 83 positive urine cultures corresponded to UTIs caused by enterobacteria (85.5%), the remaining 14.5% corresponded to infections caused by other bacterial species.

As expected, E. coli was the main enterobacteria isolated with a value of 91.6%, followed by Klebsiella sp. with 3.6%, and Klebsiella aerogenes with 1.2%. Other species of clinical importance such as Citrobacter freundii, Enterobacter cloacae and Klebsiella pneumoniae repeated 1.2% each. (See table 1).

Table 1.Percentage of uropathogens of urinary tract infections in pregnant women in the Lab. Clinical Solidarity – Lab. IESS Machala, period January – December 2022.

Enterobacteriaceae	SOLIDARITY	IESS MACHALA	TOTAL	
	LABORATORY		No.	%
<i>Escherichia coli</i>	57	19	76	91.6
<i>Klebsiella sp</i>	3	0	3	3.6

<i>Klebsiella pneumoniae.</i>	1	0	1	1.2
<i>Citrobacter freundii</i>	1	0	1	1.2
<i>Enterobacter cloacae</i>	1	0	1	1.2
<i>Klebsiella aerogenes</i>	0	1	1	1.2
TOTAL	63	20	83	100

In total, 5 results were obtained for ESBL-positive enterobacteria; of which, 4 correspond to *E. coli*, 3 obtained in the Solidario Lab. and 1 in the IESS laboratory. The other positive result belongs to *Klebsiella aerogenes* obtained in the IESS Lab. (See table 2).

Table 2.ESBL resistance mechanism of urinary tract infections in pregnant women at the Lab. Clinical Solidarity – Lab. IESS Machala, period January – December 2022.

			ESBL* Cross-tabulation of Enterobacteriaceae species						
			Enterobacteriaceae						
			<i>E. coli</i>	<i>Klebsiella sp.</i>	<i>Citrobacter freundii</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella aerogenes</i>	Total
BLEE	Negative	Count	72	3	1	1	1	0	78
		% within ESBL	92.3%	3.8%	1.3%	1.3%	1.3%	0.0%	100.0%
BLEE	Positive	Count	4	0	0	0	0	1	5
		% within ESBL	80.0%	0.0%	0.0%	0.0%	0.0%	20.0%	100.0%
Total		Count	76	3	1	1	1	1	83
		% within Enterobacteriaceae	91.6%	3.6%	1.2%	1.2%	1.2%	1.2%	100.0%

Note:Solidarity Lab – IESS Machala Lab, period January – December 2022.

Regarding the antimicrobial susceptibility profile, the 2 species of ESBL beta-lactamase-producing enterobacteria obtained previously were considered, through which we can demonstrate that the highest percentage of resistance for *E. coli* was to cefepime 50.0%, ceftriaxone 50.0%, cephalothin 50%, trimethoprim + sulfamethoxazole 40.0%, fosfomicin 25.0%, nitrofurantoin 20.0%, gentamicin 20.0%, ciprofloxacin 20.0%, norfloxacin 20.0%.

In contrast, *Klebsiella aerogenes* showed higher percentages for cephalothin 50.0% and nitrofurantoin 20.0%. (See Table 3).

Table 3.Antimicrobial susceptibility profile of the main ESBL beta-lactamase-producing enterobacteria found in urinary tract infections in pregnant women at the Lab. Clínica Solidario – Lab. IESS Machala, period January – December 2022.

ANTIBIOTICS	BLEE
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		<i>E. coli</i>	<i>Klebsiella aerogenes</i>
CRO	S	0.0 % (0)	50.0 % (1)
	R	50.0 % (1)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
FEP	S	0.0 % (0)	50.0 % (1)
	R	50.0 % (1)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
CIP	S	60.0 % (3)	20.0 % (1)
	R	20.0 % (1)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
CN	S	60.0 % (3)	20.0 % (1)
	R	20.0 % (1)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
F	S	40.0 % (2)	0.0 % (0)
	R	20.0 % (1)	20.0 % (1)
	Yo	20.0 % (1)	0.0 % (0)
NOR	S	60.0 % (3)	20.0 % (1)
	R	20.0 % (1)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
SXT	S	40.0 % (2)	20.0 % (1)
	R	40.0 % (2)	0.0 % (0)
	Yo	0.0 % (0)	0.0 % (0)
FF	S	25.0 % (1)	25.0 % (1)
	R	25.0 % (1)	0.0 % (0)
	Yo	25.0 % (1)	0.0 % (0)
KF	S	0.0 % (0)	0.0 % (0)
	R	50.0 % (1)	50.0 % (1)
	Yo	0.0 % (0)	0.0 % (0)

CRO: ceftriaxone, FEP: cefepime, CIP: ciprofloxacin, CN: gentamicina, F: nitrofurantoin, NOR: norfloxacin, SXT: trimethopin sulfamethoxazole, FF: fosfomicin, KF: cephalothin.

Discussion

Urinary tract infection (UTI) in pregnant women is currently a serious problem that constantly affects many Latin American women, causing in turn several diseases of clinical importance such as gestational hypertension, anemia, chronic kidney disease, preterm births, pyelonephritis, cystitis and stillbirth.(17,18)if not treated in time.

The study demonstrated the presence of different uropathogens in pregnant women who were treated at the Microbiology Laboratory of the IESS Machala General Hospital and the Solidarity Clinical Laboratory of Machala, whose ages ranged between 20 and 30 years of age, with a confirmed diagnosis of urinary tract infection. This study identified that the 85.5% (83/97) of positive urine cultures causing UTI belonged to the Enterobacteriaceae group. This finding is in agreement with several studies carried out in Ethiopia and Somaliland, where gram-negative bacteria prevail with 77.8% and 71% respectively.(19,20); However, although gram-positive bacteria are also involved in the appearance of UTI, it is important to mention that gram-negative aerobic bacilli

originating in the gastrointestinal tract are the main causes of UTI in pregnant women.(20,21).

The most dominant ESBL-producing uropathogenic species in this study were *Escherichia coli*(91.6%) and *Klebsiella aerogenes* (1.2%). According to epidemiological data, in other countries *E. coli* was also considered as the main ESBL-producing enterobacteria causing UTI in pregnant women, but with different proportions in various countries such as South Africa with 49.9%.(22), Ethiopia with 47.8%(20), Ghana with 47.0%(23)and Pakistan with 37.3%(24)In Tanzania, the species *Klebsiella aerogenes* was the second most prevalent uropathogen, accounting for up to 23.6% of isolates.(25), findings that agree with those of this study; however, these differences with respect to the prevalence rate of each country are due to differences between environmental conditions, characteristics of the population studied (different culture, eating-social habits and level of personal hygiene) and applied methodologies.(26). Other ESBL-producing enterobacteria in this study were *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella pneumoniae* with results similar to research carried out in southern Ethiopia(20).

Based on the above, it is important to indicate that *E. coli* is the main ESBL-producing uropathogen with greater dominance, this is because it has toxins, fimbriae, pili and adhesins that help it bind to the uroepithelium, which give protection to the bacteria in the elimination of urine and help the proliferation of bacteria and entry into the uroepithelial tissue.(27). Furthermore, the *Klebsiella aerogenes* species has been considered as one of the emerging-predominant species to be acquired in a community, due to its inherent-emerging virulence factors, including its capsule, siderophore, lipopolysaccharide, fimbriae 1-3, biofilm formation and antimicrobial resistance.(28).

The antimicrobial resistance profile in this study for *E. coli* showed moderately high resistance (50%) to antibiotics belonging to certain cephalosporin groups, including cephalothin (first generation), ceftriaxone (third generation) and cefepime (fourth generation), each drug with 50% resistance for *E. coli*; while *Klebsiella aerogenes* showed resistance mostly to cephalothin (cephalosporin - first generation) with 50%. These results are similar to a study conducted in the Middle East, where *E. coli* and *Klebsiella aerogenes* reached moderately high resistance to three antibiotics, including cephalothin, ceftriaxone and cefepime each with 40.65%, 41.41% and 63.72%.(29); At the national level, according to a research carried out in Ambato, it was determined that the most frequently isolated uropathogens were *E. coli* (72%) and *Klebsiella aerogenes* (5%), however, these proved to be highly resistant to other antibiotics such as amoxicillin/clavulanic acid, ampicillin and ampicillin/sulbactan.(30), which differs from the results of the antimicrobial resistance profile of this study.

In reference to the above, the MSP of Ecuador has indicated that the main antibiotics resistant to UTI in pregnant women are drugs such as amoxicillin and ampicillin (with or without beta-lactamase inhibitors and sulfa drugs), since these have resistance values at the national level that exceed those recommended globally.(31). Likewise, it is important to highlight that, when a resistance greater than 20% is obtained, it is advisable not to administer a single antibiotic as empirical treatment.(30); For this reason, according to the results of this study, it is suggested that the recommendation not to prescribe those antibiotics where a resistance greater than the proportion previously indicated (20%) has been found be adopted.

Finally, our results corroborate the importance of applying an appropriate and efficient diagnosis for UTIs; sinceThe production of ESBL is associated with decreased therapeutic responses, becoming a serious impediment to counteract UTIs by generally showing a high resistance to certain antimicrobial agents and thus causing a decrease in therapeutic options to treat UTIs in pregnant women.(32). Therefore, it is essentialimplement appropriate antibiotic treatments and jointly develop a compilation-analysis of advanced studies, applying rigorous and appropriately planned methodologies; in order to carry out a constant review-control of the figures regarding the resistance of ESBL-producing enterobacteria and thus increase the effectiveness of UTI treatment in the future by compiling effective medications, with the appropriate doses and posologies.(30).

Conclusions

- The current findings reflect that there is a high prevalence of UTI in pregnant women (85.5%). *Escherichia coli* and *Klebsiella aerogenes* were the ESBL-producing enterobacteria with the highest resistance to antibiotics such as FEP, CRO and KF, each with 50% resistance. The resistance rate was considered moderately high, so these drugs cannot be used in complementary treatments to treat UTI in pregnant women; in addition, they may be related to low prices, which compared to other antimicrobial agents, cause that the antibiotics in this study identified with the highest resistance could have been more accessible. Therefore, health education, permanent control and surveillance of UTIs accompanied by constant review of the antimicrobial resistance profile are essential actions to reduce the impact of UTIs and the most resistant bacteria in pregnant women.

Conflict of interest

The authors declare that there is no conflict of interest.

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