

## The Escherichia coli Strains from Asymptomatic Women of Urinary Tract Infections

Flores-Encarnación, M.<sup>1\*</sup>, Romero Serrano D.I.<sup>1</sup>, Aguilar-Gutiérrez G.R.<sup>2</sup>, Xicohténcatl-Cortés J.<sup>3</sup>, Xicohténcatl-Palacios R.C.<sup>4</sup>, Cabrera-Maldonado C.<sup>5</sup>, Carreño-López R<sup>6</sup>

<sup>1</sup>Laboratorio de Microbiología Molecular y Celular, Biomedicina. Facultad de Medicina. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla. México.

<sup>2</sup>CISEI, Instituto Nacional de Salud Pública. Cuernavaca, Morelos. México.

<sup>3</sup>Hospital Infantil de México Federico Gómez, Ciudad de México, México.

<sup>4</sup>Facultad de Medicina Veterinaria y Zootecnia. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla. México.

<sup>5</sup>Depto. De Microbiología, Facultad de Ciencias Químicas. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla. México.

<sup>6</sup>Centro de Investigaciones Microbiológicas, ICUAP. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla. México.

**\*Corresponding Author:** Flores-Encarnación, M, Laboratorio de Microbiología Molecular y Celular, Biomedicina. Facultad de Medicina. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla.

**Abstract:** Urinary tract infections are a common public health problem around the world. The most common infectious agents producing urinary tract infections are uropathogenic *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. On the other hand, an asymptomatic bacteriuria is defined as the presence of significant bacteriuria without the symptoms of an acute urinary tract infection. In recent years, asymptomatic bacteriuria has been controversial for the administration or not of antibiotics. Apparently, the asymptomatic bacteriuria does not cause renal disease or damage. Little is known about phenotypic characteristics of uropathogenic *E. coli* strains from asymptomatic bacteriuria. The objective of this study was to determine some phenotypic characteristics of uropathogenic *E. coli* strains from asymptomatic patients with urinary tract infection.

**Keywords:** *E. coli*, asynthomatic, bacteriuria, urinary, infection, tract.

### 1. INTRODUCTION

Urinary tract infections are a common public health problem around the world (Flores-Mireles *et al.*, 2015; Foxman, 2014). Among the most common causative agents of urinary tract infections can be mentioned uropathogenic *Escherichia coli*, followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. (Foxman, 2014; Nielubowicz and Mobley, 2010). In general, 40% of women develop a urinary tract infection at some point in their life. This type of infections are more frequent in women than in men (adult women are 30 times more likely than men to develop a Urinary tract infection) and there are different factors that favor their recurrence (Foxman, 2002; Giray *et al.*, 2012; Tan and Chlebicki, 2016). It has been reported that one in three women have their first episode of urinary tract infection by the age of 24 years and they are most commonly seen in sexually active young women (Tan and Chlebicki, 2016). Patients suffering from a symptomatic urinary tract infection are commonly treated with antibiotics, however the treatments can result in long-term alteration of the normal flora of vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms (Flores-Mireles *et al.*, 2015; Kostakioti *et al.*, 2012).

In recent years, asymptomatic bacteriuria has been controversial for the administration or not of antibiotics. Asymptomatic bacteriuria is demonstrated by the isolation of bacteria in quantitative counts ( $10^5$  CFU/mL of urine) from an appropriately collected urine specimen, in an individual with no acute signs or symptoms referable to the genitourinary tract (Nicolle, 2016). It has been reported that asymptomatic bacteriuria does not cause renal disease or damage (Kodner and Gupton, 2010). Several studies in women and the paediatric population have demonstrated that treatment for asymptomatic bacteriuria increases the risk of subsequent symptomatic urinary tract infections, although the treatment of asymptomatic bacteriuria in pregnant women is recommended to reduce the frequency of preterm and low-birth-weight infants (Nicolle *et al.*, 2005; Tan and Chlebicki, 2016). On the other hand, it has been reported that pathogenicity of *E. coli* strains isolated from patients with urinary tract infection, depend of presence and expression of several virulence factors, which facilitate bacterial adhesion and infection development (Salehzadeh and Zamani, 2018). However, little is known about the *E. coli* strains present in asymptomatic women of urinary tract infections. From the above, it was of interest to determine some phenotypic characteristics of *E. coli* strains under those conditions.

## 2. MATERIAL AND METHODS

### 2.1. Biological Material and Bacterial Strains

In this study, 18 urine samples of urine from asymptomatic voluntary women (from 15 to 42 years old) of urinary tract infection were used. For the collection of urine samples, the informed consent of each woman participating in this research work was requested. Women who are pregnant, menstruating, diabetic, or treated with antibiotics did not participate in the study. Each sample of urine was analyzed using a reactive strip for urine (Multistix 10 SG Siemens, Ref. 2300) according to the manufacturer's instructions. The urine samples were seeded by cross streak in Petri dishes containing sterile urine and agar (50:50, v/v) and on Mac Conkey agar plates. The plates containing urine agar were used for counting of colony forming units (CFU/mL) and Mac Conkey agar plates for the selection of lactose positive colonies. The plates were incubated at 37°C for 24 hrs. The *E. coli* strains were identified using the microbial biochemical tests described by Fernández *et al.*, (2010). Bacterial strains were stored at -30°C in tryptic soy broth with 20% glycerol until they were used. For comparison, 14 strains of *E. coli* (previously obtained in the laboratory from patients with urinary tract infection) were used.

### 2.2. Serotyping

The identity of the group-BE *E. coli* strains was confirmed by serotyping using a polyvalent antiserum (Serobac Antisuero *E. coli* E2, Biorad), including O86:K61, O112:K66, O128:K67, O119:K69, O125:K70, O126:K71 and O124:K72 serotypes. The assay was performed according to the manufacturer's instructions. For this, an *E. coli* colony was resuspended in 50 µL of isotonic saline on a slide and 50 µL of the antiserum (diluted 1:3) was added. It was mixed for 1 minute and observed under a microscope at 40x. Aggregate formation indicated a positive result for bacterial strains.

### 2.3. Measurement of Biofilm

The quantification of biofilm production was performed according to the modified method described by Stepanovic *et al.*, (2004). For that, each strain of isolated *E. coli* was pre-culture in LB broth for 24 hour at 37°C. The optical density at 560 nm was adjusted to 0.1. Then, a total of 125 µL of each bacterial pre-culture was inoculated in 5 mL of fresh tryptic soy broth. From each bacterial suspension,  $1 \times 10^6$  cell were used in sterile 96 well plates and they were incubated at 37°C during 96 hours in humid chamber.

To dye the biofilm, it proceeded to delete the liquid contents of plate wells and 250 µL of 0.1% crystal violet was added for 20 minutes. The violet crystal was removed from each well and the excess was washed using distilled water. The optical density was read spectrophotometrically at 595 nm. All assays were repeated in quadruplicate.

### 2.4. Yeast Cell Agglutination

The capacity of bacteria to express type I fimbria was assessed by their ability to agglutinate *Saccharomyces cerevisiae* cells according to the modified method described by Korhonen (1979) and Staerk *et al.*, (2016). For this, a culture of *E. coli* strains was made in LB broth containing 0.2% arabinose and incubated at 37°C for 24 hours. On the other hand, *S. cerevisiae* was grown in

YPD broth (containing  $g L^{-1}$ : 10 g yeast extract, 20 g peptone and 20 g dextrose) and allowed to incubate at 30°C for 24 hours with agitation of 200 r.p.m. Then, 1 mL of *S. cerevisiae* culture and 1 mL of *E. coli* culture were mixed and incubated at 37°C for 10 minutes. The mixture was spread on a slide and a Gram stain was performed. Yeast cell agglutination was observed under a microscope at 40x.

### **2.5. Effect of Mannose in *E. coli*-yeast Cell Agglutination**

To verify the existence of type I fimbria in *E. coli* strains, the test of inhibition with mannose was made according to the method reported by Ruggieri *et al.*, (1985). For this, a solution of 10% mannose in phosphate buffered saline (PBS, containing  $g L^{-1}$ : 11.5 g  $Na_2HPO_4$ , 2.0 g  $KH_2PO_4$ , 80.6 g NaCl, 2.2 g KCl, pH 7.4) was used. Then, 1 mL of *E. coli* suspension and 1 mL of solution of 10% mannose were mixed and incubated at 37°C for 5 minutes. 1 mL of *S. cerevisiae* culture was added and incubated at 37°C for 5 minutes. The mixture was spread on a slide, a Gram stain was performed and agglutination inhibition was observed under a microscope at 40x.

### **2.6. Antimicrobial Susceptibility Testing**

The *in vitro* susceptibility of *E. coli* strains was determined by the disk diffusion modified method according to Bauer *et al.*, (1966). For that, bacterial strains were scattered on nutrient agar plates and discs with antibiotics were used: trimethoprim/sulfamethoxazole (25 $\mu$ g), gentamicin (10 $\mu$ g), bacitracin (130 $\mu$ g), carbenicillin (100 $\mu$ g), erythromycin (15 $\mu$ g), amikacin (30 $\mu$ g), tetracycline (30 $\mu$ g) and doxycycline (30 $\mu$ g) (BD BBL, Sensi-Disc). The bacterial culture was incubated overnight at 37°C during 24 hours. After twenty-four hours proceeded to make the measurement of growth inhibition and it proceeded to compare the results with the parameters of sensitivity and resistance following the rules of Clinical and Laboratory Standards Institute.

## **3. RESULTS**

As mentioned earlier, 18 urine samples from asymptomatic voluntary women of urinary tract infection collected. Each urine sample was analyzed using a reactive strip for urine. The results obtained are shown in Table 1. As you can see, all the urine samples analyzed indicated the presence of bacteria and leukocytes (marked as + or ++). The density of the urine samples was from 1,025 to 1,030 and the pH ranged from 6.0 to 6.5. In the majority of urine samples, the presence of erythrocytes, proteins and nitrites was negative, while in all samples the presence of glucose was negative. The above data indicated the presence of bacteria in the urine samples analyzed. To isolate the bacteria present in the urine samples (lactose-positive) and quantify the amount of them, the urine samples were cultured on urine agar plates and Mac Conkey agar. As mentioned earlier, *E. coli* strains were identified using the microbial biochemical tests. The results obtained confirmed the presence of bacteria in urine samples from asymptomatic women of urinary tract infection. The amount of bacteria present in each urine sample was determined using urine agar plates. The results are shown in Table 1. As shown in Table 1, fifteen of the urine samples contained more than 10,000 CFU/mL (10,200 to 29,000), three urine samples contained smaller amounts of bacteria (5,300 to 9,800 CFU/mL). After performing isolation and identification of lactose-positive bacteria, in this study eighteen *E. coli* strains were recovered from asymptomatic women of urinary tract infection. Then, the identity of the group-B *E. coli* strains was confirmed using a polyvalent antiserum including different serotypes, as it mentioned in Materials and Methods. This serotyping test was carried out for both the *E. coli* strains from asymptomatic patients and *E. coli* strains from patients with urinary tract infection. The results are shown in Fig. 1. As shown in Fig. 1A, a positive agglutination to group B antiserum in 44% of *E. coli* strains from asymptomatic patients with urinary tract infection was observed, while the 56% of *E. coli* strains did not agglutinate in the presence of tested antiserum. These results suggested the presence of *E. coli* strains other than group B. The most strains of *E. coli* strains from symptomatic patients with urinary tract infection showed positive agglutination to group B antiserum (Fig. 1B). Thus, 77% of the strains of *E. coli* resulted positive to agglutination for group B antiserum and 23% did not agglutinate. This minority of *E. coli* strains was not group B. However, the identity of the strains was checked by microbiological methods as mentioned in Materials and Methods (data not shown).

On the other hand, the biofilm production was determined using sterile 96 well plates, 0.1% crystal violet and reading optical density at 595 nm. The results are shown in Fig. 2. As seen in Fig. 2, both

strains of *E. coli* from asymptomatic and symptomatic patients of urinary tract infections formed biofilm, which was expected because the bacteria develop naturally forming biofilm. To determine the capacity of *E. coli* strains to express type I fimbria, the cultures were made in LB broth containing 0.2% arabinose and then they were mixed with *S. cerevisiae* and incubated at 37°C for 10 minutes. The results are shown in Fig. 3. As shown in Fig. 3, the positive agglutination was observed for 89% of *E. coli* strains from asymptomatic patients with urinary tract infection, while the 11% of *E. coli* strains did not agglutinate according to the test performed. These results suggested the presence of type I fimbria in most strains of *E. coli* strains from asymptomatic patients with urinary tract infection, while all the strains of *E. coli* from symptomatic patients with urinary tract infection resulted positive to presence of type I fimbria. The results obtained supported the data of the formation of biofilm that in all strains of *E. coli* was observed. Then, assay of the inhibition with mannose was made as described in Materials and Methods. So, the agglutination inhibition was indicated of presence of type I fimbria. The results are shown in Fig. 4. As shown in Fig. 4, the agglutination inhibition was observed at 83% of *E. coli* strains from asymptomatic patients with urinary tract infection and 93% of *E. coli* from symptomatic patients with urinary tract infection. The results obtained are closely related to the presence of type I fimbria in almost all strains of *E. coli* tested, both in asymptomatic and symptomatic patients of urinary tract infection. Finally, the *in vitro* susceptibility of *E. coli* strains was determined using the disk diffusion modified method as in Materials and Methods was described. The results are shown in Table 2. As seen in Table 2, *E. coli* strains from asymptomatic patients with urinary tract infection were shown on lines 1 to 18, while strains of *E. coli* from symptomatic patients were shown on lines 19 to 31. In the strains of *E. coli* from asymptomatic patients of urinary tract infection there was a greater number of cases of sensitivity to different antibiotics, however in the strains of *E. coli* from symptomatic patients, a greater number of cases of resistance to antibiotics was observed. So, with trimethoprim/sulfamethoxazole, the majority of strains of *E. coli* from symptomatic patients of urinary tract infection were resistant (73%); 56% of *E. coli* strains were resistant to this antibiotic in asymptomatic patients (Table 2). Similar results were observed using gentamicin (93% and 78%) from symptomatic and asymptomatic patients of urinary tract infection, respectively. Using erythromycin and amikacin, 86% approximately of *E. coli* strains were resistant to antibiotics in symptomatic and asymptomatic patients of urinary tract infection. With tetracycline, 90% the *E. coli* strains were resistant in both patients. Using carbenicillin and doxycycline, all *E. coli* strains were resistant (100%) in patients symptomatic of urinary tract infection. In *E. coli* strains of asymptomatic patients, a high proportion of resistance to the antibiotics tested was also observed.

**Table1.** General characteristics of samples analyzed with reactive strips for urine.

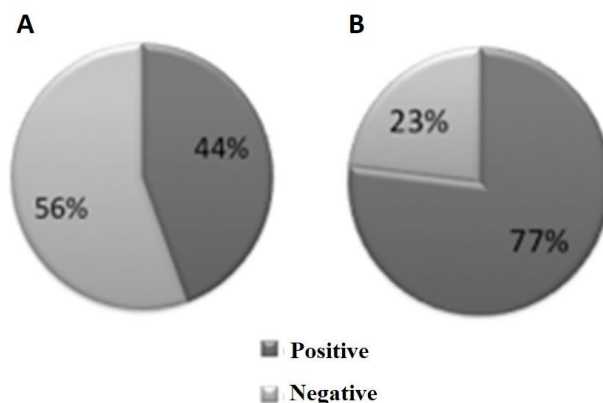
Sample	Glucose	Nitrites	Protein	pH	Density	Bacteria	Leukocytes	Erythrocytes	CFU/mL
1	Negative	Negative	Negative	6.0	1.030	Positive	2+	Negative	15,500
2	Negative	Negative	Negative	6.0	1.050	Positive	2+	Negative	26,200
3	Negative	Negative	Positive	6.0	1.030	Positive	2+	Negative	15,700
4	Negative	Positive	Negative	6.5	1.030	Positive	1+	Negative	10,200
5	Negative	Negative	Negative	6.0	1.030	Positive	2+	Negative	19,800
6	Negative	Negative	Negative	6.0	1.030	Positive	2+	Negative	20,400
7	Negative	Negative	Negative	6.0	1.030	Positive	2+	Negative	5,300
8	Negative	Negative	Negative	6.5	1.025	Positive	3+	Negative	8,900
9	Negative	Negative	Negative	6.0	1.030	Positive	2+	Negative	17,200
10	Negative	Negative	Negative	6.0	1.030	Positive	1+	Negative	15,700
11	Negative	Negative	Positive	6.0	1.030	Positive	3+	Negative	9,800
12	Negative	Positive	Negative	6.5	1.030	Positive	3+	Negative	29,000
13	Negative	Negative	Positive	6.0	1.030	Positive	1+	Negative	27,600
14	Negative	Negative	Negative	6.5	1.025	Positive	3+	Negative	24,800
15	Negative	Negative	Negative	6.0	1.030	Positive	3+	Negative	18,732
16	Negative	Positive	Negative	6.0	1.030	Positive	1+	Negative	15,200
17	Negative	Negative	Negative	6.5	1.030	Positive	2+	Negative	10,900
18	Negative	Negative	Positive	6.0	1.030	Positive	2+	Positive	15,300

**Table2.** The *in vitro* susceptibility of *E. coli* strains to antibiotics.

<i>E. coli</i> strain	Sxt	Gm	Bc	Cb	Em	Ak	Tc	Dc
1	MR	S	R	R	R	R	MS	MS
2	S	R	E	S	R		R	R
3	S	S	R	S	S	S	S	S
4	S	MR	R	R	R	MR	R	R
5	S	R	R	R	R	S	R	R
6	S	MR	R	S	R	MR	S	S
7	R	S	R	R	R	R	R	R
8	R	R	MR	R	R	R	R	R
9	R	R	MR	R	R	R	R	R
10	R	MR	R	S	R	R	R	R
11	S	R	R	S	R	S	R	R
12	S	MR	R	R	S	R	R	R
13	R	S	R	R	R	R	R	R
14	R	MR	R	R	R	R	R	R
15	R	R	R	R	R	MR	R	R
16	R	R	R	R	R	MR	R	R
17	R	R	R	R	R	MR	R	R
18	S	R	R	R	R	R	MR	MR
19	R	R	R	R	R	R	R	R
20	S	R	MR	R	R	R	S	R
21	S	R	R	R	R	R	R	R
22	S	R	R	R	R	S	R	R
23	R	MR	R	R	R	MR	R	R
24	R	R	R	R	R	R	R	R
25	R	MR	R	R	S	R	R	R
26	R	MR	R	R	S	R	R	R
27	R	R	R	R	R	R	R	R
28	R	MR	R	R	R	R	R	R
29	R	MR	R	R	R	R	R	R
30	R	S	MR	R	R	S	MR	R
31	R	R	MR	R	R	R	R	R

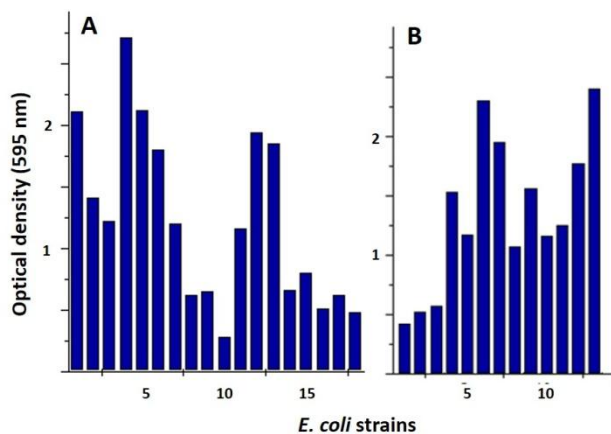
R, S, MR- Resistant, Sensitive; Moderately resistant.

Sxt-Trimethoprim/Sulfamethoxazole; Gm-Gentamicin; Bc- Bacitracin; Cb- Carbenicillin; Em- Erythromycin; Ak- Amikacin; Tc- Tetracycline; Dc- Doxycycline.

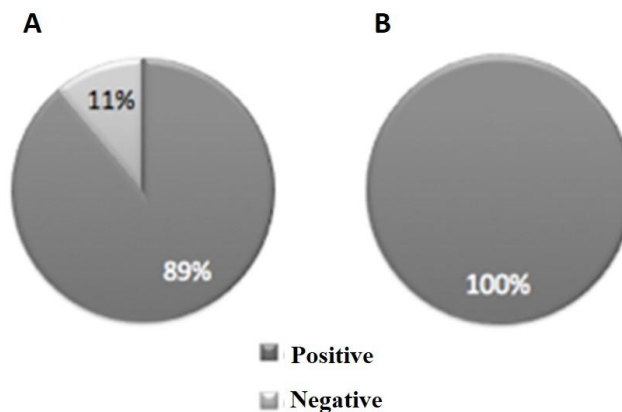


**Fig1.** The serotyping of *E. coli* strains using a polyvalent antiserum. A. *E. coli* strains from asymptomatic patients with urinary tract infection; B. *E. coli* strains from symptomatic patients with urinary tract infection.

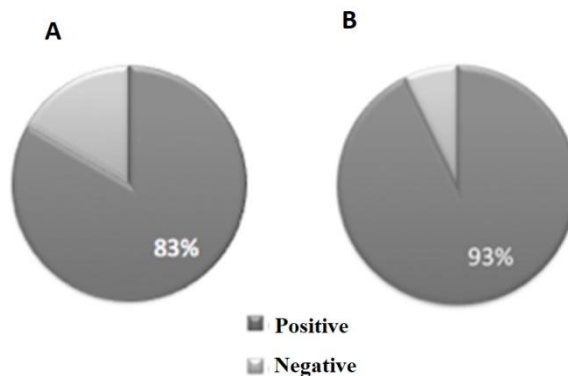




**Fig2.** The biofilm production by strains of *E. coli* from asymptomatic and symptomatic patients of urinary tract infections. A. Biofilm produced by *E. coli* strains from asymptomatic patients with urinary tract infection; B. Biofilm produced by *E. coli* strains from symptomatic patients with urinary tract infection.



**Fig3.** The existence of type I fimbria in *E. coli* strains. A. *E. coli* strains from asymptomatic patients with urinary tract infection; B. *E. coli* strains from symptomatic patients with urinary tract infection.



**Fig4.** The assay of the inhibition with mannose. A. *E. coli* strains from asymptomatic patients with urinary tract infection; B. *E. coli* strains from symptomatic patients with urinary tract infection.

#### 4. DISCUSSION

*E. coli* strains causing disease outside the gastrointestinal tract have been named “extraintestinal pathogenic *E. coli*” (ExPEC). Uropathogenic *E. coli* is the most common ExPEC pathogen in humans (Johnson and Russo, 2005; Lloyd *et al.*, 2007). So, uropathogenic *E. coli* strains isolated from urinary tract infection have been characterized by the specific virulence factors closely related with colonization and persistence of bacteria (Giray *et al.*, 2012). However, there is not much information regarding to characterization of uropathogenic *E. coli* strains isolated from asymptomatic bacteriuria. Asymptomatic bacteriuria, also called asymptomatic urinary infection, is the isolation of bacteria in quantitative counts consistent with growth within the bladder or kidneys from an appropriately collected urine specimen in an individual with no acute signs or symptoms referable to the genitourinary tract (Nicolle *et al.*, 2005; Nicolle, 2016). For this, the interest of present work was to determine some phenotypic characteristics of *E. coli* strains isolated from asymptomatic bacteriuria. So, 18 urine samples from asymptomatic voluntary women of urinary tract infection were characterized. As observed in Table 1, the biochemical values (glucose, nitrites, proteins, pH, density, erythrocytes) were found for each urine sample in permitted ranges. However, the presence of bacteria was positive in all samples; most urine samples contained more than 10,000 CFU/mL (10,200 to 29,000) and a few contained smaller amounts of bacteria (5,300 to 9,800 CFU/mL). Traditionally, it has been considered that the presence in urine of 100,000 or more bacteria/mL represents a significant bacteriuria, indicative of urinary tract infection (Kass, 1957). However, this criterion is only applicable to certain population groups and can not currently be considered an absolute criterion, for example: in young women, when starting sexual activity, can increase the prevalence (up to 5%) and the possibility of developing symptomatic urinary tract infections (De Cueto, 2005; Hooton *et al.*, 2000). Currently, the "real" presence of any number of bacteria in urine can represent a urinary tract infection, when there are specific symptoms and pyuria (De Cueto, 2005; Foxman, 2014). In the present study, women patients did not present specific symptoms of urinary tract infection, so it was an asymptomatic bacteriuria. The asymptomatic bacteriuria has caused many controversies about its clinical importance, such as the influence on the state of health, its possible progression towards the appearance of symptomatic urinary tract infections, the potential existence of an anatomical malformation in the urinary system, its relationship with the production of renal scars and, whether patients should be treated with antibiotics or not (García-Nieto *et al.*, 2011; Nicolle, 2006; Nicolle, 2016). Some authors have proposed that administration of antibiotics in asymptomatic bacteriuria do not reduce the rates of subsequent complication and paradoxically, may increase the risk of urinary tract infection (Cai *et al.*, 2015; Cortes-Penfield *et al.*, 2017). Furthermore, unnecessary antibiotic treatment is associated with acquisition of drug resistant pathogens and other drug-related adverse events (Cai *et al.*, 2015; Wagenlehner *et al.*, 2005; Werner *et al.*, 2011). Guidelines from the Infectious Diseases Society of America recommend treating of urinary tract infection only in pregnant women or immediately prior to a urologic procedure likely to involve mucosal (Cortes-Penfield *et al.*, 2017; Nicolle *et al.*, 2005). As it was mentioned before, the identity of *E. coli* strains was confirmed using a polyvalent antiserum for B-group *E. coli* (including different serotypes: O86:K61, O112:K66, O128:K67, O119:K69, O125:K70, O126:K71 and O124:K72). So, 44% *E. coli* strains from asymptomatic patients with urinary tract infection agglutinated in the presence of antiserum; 23% of the strains of *E. coli* from symptomatic patients with urinary tract infection resulted negative to agglutination for antiserum. However, identity of the strains was checked by microbiological methods as mentioned before. It has been reported that virulence factors associated with uropathogenic *E. coli* pathotype include several combinations of certain somatic and capsular antigens, ability to adhere to the uroepithelial cells by fimbrial and afimbrial adhesins, production of toxins, siderophores or iron acquisition systems, serum resistance mechanisms and invasions (Regua-Mangia *et al.*, 2010; Yamamoto, 2007). Further, *E. coli* strains that cause the majority of urinary tract infections are thought to represent only a subset of the strains that colonize the colon (Mobley *et al.*, 2009). According to the above, in the present study a large proportion of *E. coli* strains from asymptomatic patients as symptomatic of urinary tract infection could have their origin from serotypes associated with diarrheagenic *E. coli* pathotypes as has been reported by some authors (Abe *et al.*, 2008; Regua-Mangia *et al.*, 2010). Certain uropathogenic *E. coli* strains may carry diarrheagenic *E. coli* virulence properties, mostly associated to enteroaggregative *E. coli* pathotype. This finding raises the possibility that at least some faecal enteroaggregative *E. coli* strains might represent potential uropathogens and

that alternatively, certain uropathogenic *E. coli* strains may have acquired enteroaggregative *E. coli* properties, becoming a potential cause of diarrhoea (Abe *et al.*, 2008).

The uropathogenic *E. coli* strains from asymptomatic patients with urinary tract infection were characterized by their ability to produce biofilm, fimbriae and their sensitivity to different antibiotics. The results shown that both strains of *E. coli* from asymptomatic and symptomatic patients of urinary tract infections formed biofilm, which was expected because the bacteria develop naturally forming biofilm (Costerton *et al.*, 1995; Costerton, 1999; Eberly *et al.*, 2017). Biofilm formation significantly enhances resistance towards removal by both natural defence mechanisms and antibiotics (Costerton *et al.*, 1999). It has been reported that biofilm formation by *E. coli* strains in asymptomatic bacteriuria appears to be an important strategy used by these strains for persistence in this high-flow environment as human urinary tract (Hancock *et al.*, 2007). It has been observed also that asymptomatic bacteriuria *E. coli* strains grow fast in human urine (as it was determined in the present study) and that fast growth probably constitutes one strategy used by these strains for remaining in the urinary tract. Further, bacterial biofilms are highly resistant to removal by liquid flow forces (Hancock *et al.*, 2007; Foxman, 2002; Roos *et al.*, 2006). The human urinary tract is submitted to strong hydrodynamic forces, while adherence to urinary tract epithelium enables bacteria to resist removal by urine flow and produce infection (Hancock *et al.*, 2007). Bacterial adherence is considered an essential step in infection process and leads to colonization and invasion and host cell damage (Mulvey, 2002). In the present study, the strains of uropathogenic *E. coli* from asymptomatic patients with urinary tract infection by the presence of type I fimbria were characterized. The results shown that 89% of *E. coli* strains produced type I fimbriae, while 11% of *E. coli* strains did not produce. All strains of *E. coli* from symptomatic patients with urinary tract infection resulted positive to presence of type I fimbria. Similar results were obtained by mannose inhibition test, where it was observed that 83% of *E. coli* strains from asymptomatic and 93% of *E. coli* from symptomatic patients with urinary tract infection resulted positive to presence of type I fimbria. It has been reported that primary fimbrial adhesins associated with uropathogenic *E. coli* strains are type 1 fimbria, P fimbria and F1C fimbria (Bäckhed *et al.*, 2002; Hancock *et al.*, 2007). The type I fimbria of the uropathogenic *E. coli* strains must play an important role in the adhesion to the epithelium of the urinary tract and contribute to formation of biofilm.

Finally, the *in vitro* susceptibility of uropathogenic *E. coli* strains was determined using the disk diffusion method. The results shown that the *E. coli* strains from asymptomatic patients with urinary tract infection were most sensitive than *E. coli* strains from symptomatic patients to antibiotics tested, however the resistance was observed in all cases. 56 % and 73% of *E. coli* strains were resistant to trimethoprim/sulfamethoxazole from strains of asymptomatic and symptomatic patients of urinary tract infection, respectively. 78% and 93% were resistant to gentamicin from strains of asymptomatic and symptomatic patients of urinary tract infection, respectively. 86% of *E. coli* strains were resistant to erythromycin and amikacin in both cases. 90% of *E. coli* strains were resistant to tetracycline in both cases. 100% of *E. coli* strains were resistant to carbenicillin and doxycycline in both cases. Antibiotic resistance was observed in strains of uropathogenic *E. coli*, especially in strains from asymptomatic patients with urinary tract infection. Since these antibiotics are oral treatment options for different infectious diseases, it could explain the resistance observed in strains of *E. coli* from asymptomatic patients with urinary tract infection (Giray *et al.*, 2012).

## 5. CONCLUSION

*E. coli* is the infectious agent that is most frequently isolated in urinary tract infections. The uropathogenic *E. coli* strains have been characterized in virulence factors related with colonization and persistence in the urinary tract. Little is known about the uropathogenic *E. coli* strains from asymptomatic patient of urinary tract infections. The present study shown that these strains of uropathogenic *E. coli* had phenotypic characteristics similar to those of the strains from patients symptomatic of urinary tract infection, especially resistance to different antibiotics. That could suggest that at some time *E. coli* strains could be responsible for an infectious process and cause disease in the asymptomatic patients.

## ACKNOWLEDGEMENTS

Thank to PRODEP and Facultad de Medicina-BUAP for the facilities provided for the development of this work.



## REFERENCES

- [1] Abe C.M., Salvador F.A., Falsetti I.N., Vieira M.A., Blanco J., Blanco J.E., Blanco M., Machado A.M., Elias W.P., Hernandez R.T., Gomes T.A. (2008). Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic *E. coli*. *FEMS Immunol. Med. Microbiol.* 52:397-406.
- [2] Bäckhed F., Alsen B., Roche N., Angstrom J., von Euler A., Breimer M.E., Westerlund-Wikstrom B., Teneberg S. and Richter-Dahlfors A. (2002). Identification of target tissue glycosphingolipid receptors for uropathogenic, F1C-fimbriated *Escherichia coli* and its role in mucosal inflammation. *J. Biol. Chem.* 277:18198-18205.
- [3] Bauer A.W., Kirby W.M, Sherris J.C. and Turck M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45:493-496.
- [4] Cai T., Nesi G., Mazzoli S., Meacci F., Lanzafame P., Caciagli P., Mereu L., Tateo S., Malossini G., Selli C. and Bartoletti R. (2015). Asymptomatic bacteriuria treatment is associated with a higher prevalence of antibiotic resistant strains in women with urinary tract infections. *Clin. Infect. Dis.* 61:1655-1661.
- [5] Cortes-Penfield N.W., Trautner B.W. and Jump R. (2017). Urinary tract infection and asymptomatic bacteriuria in older adults. *Infect. Dis. Clin. North Am.* 31:673-688.
- [6] Costerton, J.W, Lewandowski, D.E., Cadwell, D.R., Corber, H.M. and Lappin-Scott, H. 1995. Microbial biofilms. *Annu. Rev. Microbiol.* 49: 711-745.
- [7] Costerton, J.W. 1999. Introduction to biofilms. *Int J Antimicrob Agents.* 11: 217-221.
- [8] De Cueto M. (2005). Diagnóstico microbiológico de la infección del tracto urinario. *Enferm. Infecc. Microbiol. Clin.* 23:9-14.
- [9] Eberly A.R., Floyd K.A. Beebout C.J., Colling S.J., Fitzgerald M.J., Stratton C.W., Schmitz J.E. and Hadjifrangiskou M. (2017). Biofilm formation by uropathogenic *Escherichia coli* is favored under oxygen conditions that mimic the bladder environment. *Int. J. Mol. Sci.* 18:2077-2088.
- [10] Fernández-Olmos, A., García-de la Fuente, C., Saéz-Nieto, J.A., Valdezate-Ramos, S. 2010. Métodos fenotípicos de identificación. En: Métodos de identificación bacteriana en el laboratorio de microbiología. Ed. Cercenado E. y Cantón, R. Madrid, España. pp 31-43.
- [11] Flores-Mireles A.L., Walker J.N., Caparon M. and Hultgren S.J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* 13:269-284.
- [12] Foxman B. (2002). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am. J. Med.* 113 Suppl 1A:5S-13S.
- [13] Foxman B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect. Dis. Clin. North Am.* 28:1-13.
- [14] García-Nieto V.M., González-Cerrato S., García-Rodríguez V.E., Luis-Yanes M.I., Martín-Conde L. and Pozo-García E. (2011) Bacteriuria asintomática. *Bol. Pediatr.* 51:3-10.
- [15] Giray B., Ucar F.B., Aydemir S.S. (2012). Characterization of uropathogenic *Escherichia coli* strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir. *Turk J. Med. Sci.* 42:1328-1337.
- [16] Hancock V., Ferrière L. and Klemm P. (2007). Biofilm formation by asymptomatic and virulent urinary tract infectious *Escherichia coli* strains. *FEMS Microbiol. Lett.* 267:30-37.
- [17] Hooton T.M., Scholes D., Stapleton A.E., Roberts P.L., Winter C., Gupta K., Samadpour M. and Stamm W.E. (2000). A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl. J. Med.* 343:992-997.
- [18] Johnson J.R. and Russo T.A. (2005). Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. *Int. J. Med. Microbiol. Rev.* 295:383-404.
- [19] Kass E.H. (1957). Bacteriuria and the diagnosis of infections of the urinary tract. *Arch. Intern. Med.* 100:709-714.
- [20] Kodner C.M. and Gupton TEK. (2010). Recurrent urinary tract infections in women: diagnosis and management. *Am. Fam. Physician.* 82:638-643.
- [21] Korhonen T. (1979). Yeast cell agglutination by purified enterobacterial pili. *FEMS Microbiol. Lett.* 6:421-425.
- [22] Kostakioti M., Hultgren S.J., Hadjifrangiskou M. (2012). Molecular blueprint of uropathogenic *Escherichia coli* virulence provides clues toward the development of anti-virulence therapeutics. *Virulence.* 3:592-594.
- [23] Lloyd A.L., Rasko D.A. and Mobley H.L.T. (2007). Defining genomic islands and uropathogen-specific genes in uropathogenic *Escherichia coli*. *J. Bacteriol.* 189:3532-3546.
- [24] Mobley H.L., Donnenberg M.S. and Hagan E.C. (2009). Uropathogenic *Escherichia coli*. *EcoSal Plus.* 3:2.

- [25] Mulvey M.A. (2002). Adhesion and entry of uropathogenic *Escherichia coli*. *Cel. Microbiol.* 4:257-271.
- [26] Nicolle L.E. (2016). The paradigm shift to non-treatment of asymptomatic bacteriuria. *Pathogens.* 5:38-44.
- [27] Nicolle L.E. (2006). Asymptomatic bacteriuria-review and discussion. *Inter. J. Antimicrob. Agents.* 28S:S42-S48.
- [28] Nicolle L.E., Bradley S., Colgan R., Rice J.C., Schaeffer A. and Hooton T.M. (2005). Infectious diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin. Infect. Dis.* 40:643-654.
- [29] Nielubowicz G.R., Mobley H.L. (2010). Host–pathogen interactions in urinary tract infection. *Nature Rev. Urol.* 7:430-441.
- [30] Regua-Mangia A.H., Irino K., da Silva Pacheco R., Pimentel Bezerra R.M., Santos Périssé A.R. and Martins Teixeira L. (2010). Molecular characterization of uropathogenic and diarrheagenic *Escherichia coli* pathotypes. *J. Basic Microbiol.* 50:S107-S115.
- [31] Roos V., Nielsen E.M. and Klemm P. (2006). Asymptomatic bacteriuria *Escherichia coli* strains: adhesins, growth and competition. *FEMS Microbiol. Lett.* 262:22-30.
- [32] Ruggieri M.R., Hanno P.M. and Levin R.M. (1985). Mannose inhibition of *Escherichia coli* adherence to urinary bladder epithelium: comparison with yeast agglutination. *Urol. Res.* 13:79-84.
- [33] Salehzadeh A. and Zamani H. (2018). Characterization of (Uropathogenic) *E. coli* isolated from urinary tract infections: phylogenetic typing and distribution of virulence-associated traits. *Brit. J. Biom. Sci.* 75:40-42.
- [34] Staerk K., Khandige S., Kolmos H.J., Møller-Jensen J. and Andersen T.E. (2016). Uropathogenic *Escherichia coli* express type 1 fimbriae only in surface adherent populations under physiological growth conditions. *The J. Infect. Dis.* 213:386-394.
- [35] Stepanovic S., Cirkovic I., Raning L. and Svabic-Vlahovic M. (2004). Biofilm formation by *Salmonella* sp and *Listeria monocytogenes* on plastic surface. *Lett. Appl. Microbiol.* 38:428-432.
- [36] Tan C.W. and Chlebicki M.P. (2016). Urinary tract infections in adults. *Singapore Med. J.* 57:485-490.
- [37] Wagenlehner F.M., Naber K.G. and Weidner W. (2005). Asymptomatic bacteriuria in elderly patients: significance and implications for treatment. *Drugs Aging.* 22:801-807.
- [38] Werner N.L., Hecker M.T., Sethi A.K. and Donskey C.J. (2011). Unnecessary use of fluoroquinolone antibiotics in hospitalized patients. *BMC Infect. Dis.* 11:187-193.
- [39] Yamamoto S. (2007). Molecular epidemiology of uropathogenic *Escherichia coli*. *J. Infect. Chemother.* 13:68-73.

**Citation:** Flores-Encarnación, M. et al., "The *Escherichia coli* Strains from Asymptomatic Women of Urinary Tract Infections", *International Journal of Research Studies in Biosciences (IJRSB)*, vol. 6, no. 8, pp. 7-16, 2018. <http://dx.doi.org/10.20431/2349-0365.0608002>

**Copyright:** © 2018 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.