Original Article

Virulence genes and antimicrobial susceptibility of *Escherichia coli* taken from women with vaginitis in Talca, Chile

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Abstract

Introduction: Vaginitis is one of the most common reasons women visit a gynecologist. *Escherichia coli* has been isolated from women with vaginitis, but its role as a vaginal infection aetiological agent is controversial.

This study aimed to detect virulence genes and determine the antimicrobial susceptibility of *E. coli* strains isolated from monomicrobial and polymicrobial cultures collected from women with vaginitis.

Methodology: The presence of the following virulence genes: *papC*, *hly*, *iucC*, *afa*, *fimH*, *neuC*, *sfa/foc*, *cnf1*, *usp*, and *ibeA* in two *E. coli* groups was determined by PCR. The antibacterial susceptibility of strains was tested.

Results: A higher percentage (93.3%) of isolated strains from monomicrobial cultures with virulence genes in relation to polymicrobial cultures (56.7%) was found. The most frequent virulence genes in both groups were *hly* ($p = 0.0357$), *fimH* ($p = 0.000$), and *cfn1* ($p = 0.000$). In addition, *E. coli* isolated from monomicrobial cultures showed 5 genetic combinations compared to the 10 observed in the polymicrobial cultures. An increased number of strains were sensitive to cefotaxime, moxifloxacin, and ciprofloxacin. A high resistance to trimethoprim-sulfamethoxazole was observed.

Conclusions: Most of the *E. coli* strains isolated from monomicrobial cultures and some from polymicrobial cultures showed virulence genes. A better understanding of the virulence and antibacterial susceptibility of *E. coli* strains isolated from patients with vaginitis can contribute to improved diagnosis and treatment of this disease.

Key words: *Escherichia coli*; vaginitis; virulence genes; antimicrobial susceptibility; Chile

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Introduction

Vaginitis is one of the most common reasons women visit a gynecologist [1]. Its consequences are variable and include psychological damage caused by spontaneous abortions, preterm births, preterm premature rupture of the membranes, chorioamnionitis, post-partum endometritis, pelvic inflammatory disease, post-operative infections after gynaecological surgery, and easier acquisition of HIV [2,3]. These infections constitute about 90% of the infectious diseases affecting the female genital tract. Some of the main aetiological agents of vaginitis are *Trichomonas vaginalis*, *Candida albicans, Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Neisseria gonorroheae*, *Streptococcus agalactiae, Staphylococcus aureus* and others such as *Prevotella* spp., *Porphyromonas* spp., *Bacteroides* spp., and *Fusobacterium* spp. [4].

At present, *Escherichia coli* is not considered an aetiological agent of vaginitis, with the exception of the aerobic vaginitis [5]. Recent studies have reported that this microorganism is not part of vaginal microbiome [6,7]. In this context, the isolation of this bacterial species from vaginitis should be studied. Previous studies have indicated that strains of these species might be associated with vaginitis, particularly in women with inflammation, vaginal discharge, and dyspareunia [8,9]. By incorrectly assessing *E. coli* as a vaginal microbiota member, women infected with these bacterial species are not properly diagnosed or clinically treated.

E. coli is commoly found in human and animal gastrointestinal tracts. The balance between *E. coli* and the immune system of its hosts is responsible for colonizing the intestine without significantly damaging it [10]. *E. coli* is a microorganism with a high ability to adapt to new ecosystems through its high genetic plasticity. This property has resulted in variable *E. coli* pathotypes, such as the diarrhoeagenic and uropathogenic [11]. In this context, *E. coli* strains,

inducing extra intestinal infections, have their own virulent arsenal [12-14].

To better understand the biological role of this microorganism in vaginitis, it is important to determine some main pathogenic characteristics. Furthermore, to initiate appropriate clinical treament, it is important to know its antimicrobial susceptibility. The aim of this study was to detect virulence genes and determine the antimicrobial susceptibility in *E. coli* strains isolated from monomicrobial and polymicrobial cultures taken from women infected with vaginitis.

Methodology

Sample size

Sample size calculation was determined with a confidence interval of 95% with a precision error of 5% and an estimated proportion of 6% for monomicrobial isolates of *E. coli* (group 1), and 6.5% for polymicrobial isolated (group 2) [8]. Seventy-seven samples for each group were estimated. Considering the eventual loss of 10% of the samples, the sample size was increased to 90 strains in each studied group.

Source of bacterial strains

Vaginal swabs (three swabs per patient) were taken with a speculum from the lateral and posterior vaginal fornix by a gynecologist at the Hospital of Talca between July and November 2011. The samples were processed at the Microbiological Research Laboratory at the University of Talca. *E. coli* strains were obtained from women with a clinical diagnosis of vaginitis. Patients' diagnosis included clinical history and physical examination. Samples were processed and monomicrobial and polymicrobial cultures were obtained. One hundred and eighty cultures with *E. coli* were studied and separated into two groups: 90 isolates with single *E. coli* (group 1) and 90 with *E. coli* associated to recognized aetiological agents of vaginitis (group 2) such as *Trichomonas vaginalis, C. albicans*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mobiluncus*, *Neisseria gonorroheae*, and *Streptococcus agalactiae* and four strictly anaerobic bacterial species less frequently isolated from vaginitis such as *Bacteroides fragilis, Fusobacterium nucleatum, Porphyromonas gingivalis* and *Prevotella intermedia* [15]. The inclusion criteria were: women of reproductive age, between 17 and 41 years old, nonsmokers, without a history of sexually transmitted diseases, and with only one sexual mate during the last six months. Women who had had antibiotic treatment in the six precedent months were excluded, as were those who, during the checkup, were menstruating or had diarrhea or a urinary tract infection. The study protocol was accepted by Bioethical Committee of the Universidad de Talca and the patients' consent was included and reported before clinical samples were collected [8].

Determination of virulence genes

All the *E. coli* strains were cultured at 37°C in 10 mL of LB broth (OD_{550} 2.5), centrifuged at 8000 g, and the precipitate obtained was used in the genomic DNA extraction. The Genomic DNA Purification Kit (Bio-Rad Laboratories, Hercules, CA, USA) was used in this procedure, following the manufacturer's instructions. The DNA was stored at 4°C until use. In this study, the *E. coli* virulence genes most frequently associated with extraintestinal infections were analyzed: *papC* (fimbrias P) [13], *hly* (α-haemolysin) [13], *iucC* (aerobactin) [13], *afa* (afimbrial adhesin) [13], *fimH* (type 1 pili) [13], *neuC* (K1 capsule antigen) [13], *sfa/foc* (S pili type and 1C fimbriae type) [13], *cnf1* (cytotoxic necrotizing factor) [16], *usp* (uropathogen-specific protein) [17], and *ibeA* (invasion of brain endothelium) [13]. Each virulence gene was studied in 180 *E. coli strains* during this study. The PCR primer nucleotide sequences were previously described in the above-mentioned references. The amplification methodology of the the *fimP*, *hly, iucC, afa, neuC, sfa/foc, fimH*, *usp,* and *ibeA* genes was used in accordance with the literature [18]. The amplification products were separated by electrophoresis in a 1.5% agarose gel and visualized after ethidium bromide (0.25 µg/mL) staining. A 100 bp DNA ladder (New England BioLabs Inc, Beverly, MA, USA) was used in each gel as a molecular size marker. Negative controls without virulence genes of *E. coli* strains were used and the assays were conducted twice. These strains were obtained from faecal samples of healthy women.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Merck, Darmstadt, Germany) using the disk diffusion technique described by Bauer *et al.* [19]. The antibiotic disks (Valtek, Santiago, Chile) contained ampicillin (10 µg), trimethoprim-sulfamethoxazole (25 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), cefuroxime (30 µg), cefotaxime (30 µg), doxicicline (30 µg), gentamicin (10 µg), and amikacin (30 µg). The *E. coli* strain ATCC 2992 was used as a quality control method.

Statistical analysis

The statistical correlation between the vaginal origin of the studied bacterial strains (groups 1 and 2) and the carriage of different virulence genes was estimated using the chi-squared test. A p value < 0.05 was considered statistically significant. The statistical analysis was performed using SPSS version 15.0.

Results

Association of E. coli with other microorganisms in vaginitis

E. coli strains (group 2) were associated with *T. vaginalis* (27%), *C. albicans* (43%), *C. trachomatis* (6%), *U. urealyticum* (5%), *M. hominis (*4%), *Mobiluncus* spp. (2%), *N. gonorrhoeae (*5%), *S. agalactiae* (3%), *B. fragilis* (1%), *F. nucleatum* (1%), *P. gingivalis* (1%), and *P. intermediate* (1%).

Determination of virulence genes in E. coli strains obtained from vaginitis and its genetic combinations

Table 1 shows an increased number of *E. coli* strains with virulence genes in group 1 (93.3%) compared to group 2 (56.7%). Table 2 shows that the *fimH* gene was the most frequently detected gene in group 1 and that the *hly* gene was the most frequently detected gene in group 2. The less common gene was *ibeA* in both strains groups. The *afa* gene was not observed in the two groups of the studied strains. None of the analyzed virulence genes (data not shown) were detected in the three *E. coli* strains isolated from healthy women. The most frequent virulence genes in both groups (1 and 2) were *hly* (p = 0.0357), *fimH* (p = 0.000), and *cfn1* (p = 0.000).

It was observed that group 1 showed 5 genetic combinations in comparison to the 10 combinations detected in group 2. The most frequent strain combination of group 1 was detected in 26 strains (*papC- hly+ iucC+ afa- neuC+ fimH+ sfa/foc- cnf1+ usp- ibeA-*) and the most frequent strain combination in group 2 was detected in 17 strains (*papC- hly+ iucC- afa- neuC- fimH+ sfa/foc+ cnf1+ usp- ibeA-*) (data not shown).

E. coli *strain antimicrobial susceptibility*

Both studied *E. coli* strain groups showed a similar antimicrobial susceptibility pattern, and a larger number of strains sensitive to cefotaxime, moxifloxacin, and ciprofloxacin was observed. Additionally, a high number of strains resistant to trimethoprim-sulfamethoxazole was found (Table 3).

Discussion

The virulence genes present in 180 *E. coli* strains taken from monomicrobial (group 1) and polymicrobial (group 2) cultures were analyzed in this research, as well as three *E. coli* strains collected from healthy women. The strains' susceptibility was also tested. The most common genes observed in both groups were *fimH*, *hly*, and *cnf1*. These genes were detected in a higher proportion in the bacterial strains isolated from monomicrobial cultures. The strains with the genes *hly* and *fimH* exhibited an interesting relation with uropathogenic *E. coli.* These genes gave hemolytic and adhesion properties through type 1 fimbriae. Additionally, an interesting association between *cnf1* and *papC* genes was observed, mainly found in *E. coli* strains isolated from extraintestinal infections in humans and animals [14,20]. It has been shown that *cnf1* and *hly* genes are in the same pathogenicity island, facilitating simultaneous spreading. This implies a high association between these genes [21]. Another study showed that the afimbrial adhesin coded by the *afa* gene was found in a limited number of strains and was unusual in extraintestinal strains [22]; this is consistent with our results. The capsular antigen K1, coded by the *neuC* gene, is associated with neonatal meningitis [14]. The *E. coli* strains causing meningitis belong to a limited number of serogroups O and 80% of these have this capsular antigen [23]. The *ibeA* gene, associated with the brain epithelium invasion, was detected in a reduced number of *E. coli* strains of both groups analyzed in this research. This information is particularly relevant for pregnant women infected with *E. coli* strains with both genes, as the risk that their newborns may suffer meningitis or other neurological problems increases [13,24]. The physiological conditions of different human ecosystems put a strong selective pressure on the colonizing microorganisms, inducing changes in the subdominant *E. coli* population, so these species better adapt to these ecosystems during the infection [13]. Furthermore, it is possible that during the co-evolution process with humans, *E. coli* strains isolated from vaginitis might slowly turn into a new pathogenic variety; however, it may also be possible that the studied genes may not be functional in the different physicochemical conditions of the vaginal habitat. It should be also noted that the studied bacteria was isolated from women suffering vaginitis, suggesting that most of these genes might be active.

Table 1. Number and percentage of *E. coli* strains that carried virulence genes

	Virulence genes	
E. coli strains[a]	Number	Percentage
Group 1 (n = 90)	84	93.3%
Group 2 (n =90)	51	56.7%

[a]*E. coli* strains isolated from monomicrobial cultures (group 1) and polymicrobial cultures (group 2)

Table 2. Frequency of detection of different virulence genes of *E. coli* strains isolated from vaginitis

Virulence gene[a]	Group 1[b]		Group 2[c]		
	N	%	N	%	P
papC	26	28.9	17	18.9	0.1157
hly	57	63.3	43	47.8	0.0357
iucC	39	43.3	33	36.7	0.3613
afa	0	0	0	0	---------
neuC	34	37.8	22	24.4	0.0534
fimH	58	64.4	42	46.7	0.0000
sfa/foc	50	55.6	35	38.9	0.0251
cnf1	71	78.9	39	43.3	0.0000
usp	26	28.9	15	16.7	0.0506
ibeA	13	14.4	8	8.9	0.2457

[a] Type P pili (*papC*), α-hemolysin (*hly*), aerobactin (*iucC*), afimbrial adhesin (*afa*), K1 capsule antigen (*neuC*), type 1 fimbriae (*fimH)*, S fimbriae and F1C fimbriae (*sfa/foc*), cytotoxic necrotizing factor (*cnf1)*, uropathogen specific protein (*usp*), invation brain endothelium (*ibeA*).
[b] Group 1*: E. coli* strains obtained from monomicrobial cultures
[c] Group 2: *E. coli* strains obtained from polymicrobial cultures

Table 3. Antimicrobial susceptibility of *E. coli* strains isolated from vaginitis

	Number of *E. coli* strains								
Antimicrobial	amp	sxt	cip	mox	cef	cft	dox	gen	ami
				Group 1[a] (n = 90)					
S	69	46	86	88	82	88	73	75	79
R	21	44	4	2	8	2	17	15	11
				Group 2[b] (n = 90)					
S	66	50	83	85	86	89	78	79	82
R	24	40	7	5	4	1	12	11	8

S and R: sensitive and resistant
[a] *E. coli* strains obtained from monomicrobial cultures
[b] *E. coli* strains obtained from polymicrobial cultures
amp: ampicillin (10 µg); sxt: trimethoprim-sulfamethoxazole (25 µg); cip: ciprofloxacin (5 µg); mox: moxifloxacin (5 µg); cef: cefuroxime (30 µg); cft: cefotaxime (30 µg); dox: doxicicline (30 µg); gen: gentamicin (10 µg); ami: amikacin (30 µg)

Interestingly, *E. coli* strains isolated from mono and polymicrobial cultures showed similar antimicrobial susceptibilities. Most of the *E. coli* strains were sensitive to fluoroquinolones – specifically cefotaxime. These outcomes are similar to those found in a previous study [8], but differ from the results of studies of other *E. coli* strains isolated from extraintestinal environments other than the vagina [25,26]. These results are very important at the time the physician begins appropriate antimicrobial treatment.

Conclusions

Most of the *E. coli* strains isolated from monomicrobial cultures – and some from polymicrobial cultures – showed virulence genes. A better understanding of the virulence and antibacterial susceptibility of *E. coli* strains isolated from patients with vaginitis can contribute to improved diagnosis and treatment of this disease. Thus, *E. coli* strains with a high capacity to cause vaginitis can be early detected and treated. The vaginitis caused by *E. coli* in a pregnant woman might have dangerous consequences for her and her fetus.

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