JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Commensal and virulent *Escherichia coli* strains of vaginal origin are reservoirs of resistance cassettes in class 1 integrons

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Abstract

Introduction: Antimicrobial resistance in *Escherichia coli,* one of the causal agents of aerobic vaginitis, leads to the persistence of the infection. The investigation of integrons acquires relevance, since they are elements that are responsible for the acquisition of resistance to antibiotics. The aim of this work was to describe the structural diversity of class 1 integrons in virulent and commensal strains of *E. coli* isolated from patients with vaginal infection.

Methodology: Ninety-two strains of *E. coli* were isolated from patients with aerobic vaginitis. Resistance profile against 19 antibiotics and class 1 integrons were detected by PCR. The identity and arrangement of cassettes was determined by sequencing. ERIC-PCR assays were carried out in strains with identical arrays. Finally, genotyping by Clermont algorithm and serotyping were performed. Seventeen strains showed integrons located in plasmids.

Results: Ten different gene cassette arrays were identified in 30 strains of *E. coli*. Cassettes corresponding to genes coding for adenylyltransferases (*aadA)*, dihydrofolate reductases (*dfrA*), and oxacillinases (*bla*$_{OXA}$) were detected. Array *dfrA17-aadA5* was predominantly prevalent over the other arrays identified. Phylogenetic group A was the most predominant, followed by group B2 and D.

Conclusions: This study demonstrates the presence of *E. coli* of vaginal origin carrying class 1 integrons, which are main genetic elements of capture of resistance genes, with the possibility of capturing new resistance cassettes. These evidences should serve for the modification of protocols in the diagnosis and treatment of aerobic vaginitis, and the development of policies for the rational use of antimicrobials.

Key words: *Escherichia coli*; Class 1 integrons; antimicrobial resistance.

J Infect Dev Ctries 2020; 14(1):48-58. doi:10.3855/jidc.11940

(Received 19 August 2019 – Accepted 12 December 2019)

Introduction

The majority of public health problems worldwide has been generated as a result of the appearance and resurgence of infectious diseases. Many of the "new diseases" are not due to newly identified pathogens or pathogens, since there are conditions in the population, which favor their development, and thus, can become an emergency situation with an impact on Public Health [1]. Within this type of diseases, there are vaginal infections, where members of the *Enterobacteriaceae* family have been implicated in vaginal infectious processes [2]. Vaginal infections are usually the result of alterations of the vaginal microbiota, where lactobacilli are displaced due to the proliferation of other bacteria, including potentially pathogenic; therefore, the clinical diagnosis of these infectious

processes is fundamental to provide a therapy according to the etiological agent detected in the laboratory, and not only to recognize the classical etiological agents (infection by *Trichomonas vaginalis*, *Candida albicans*, and pathogens causing bacterial vaginosis (BV)), but also to provide therapy [3,4]. Furthermore, new causes of vaginal infection, such as aerobic vaginitis (AV) have been described in recent years, and there is evidence that this condition is associated with a higher risk of premature birth than BV [5]. It has been shown that due to the anatomical proximity of the "anorectal/vagina" region, some enterobacteria can act as uropathogens, and can be associated with cases of bacterial vaginosis, *Escherichia coli* being one of the main etiological agents of cases of aerobic vaginitis. However, the identification and report of these microorganisms are usually discarded in diagnostic laboratories, since they are considered contaminating or transient biota, even when the microbiological culture is abundant. The *Enterobacteriaceae* family is constituted by heterogeneous Gram-negative bacteria. In this group are *E. coli, Shigella* spp., *Klebsiella* spp*., Enterobacter* spp*., Serratia* spp*., Hafnia* spp*., Citrobacter* spp*., Proteus* spp., *Salmonella* spp., *Yersinia* spp., *Providencia* spp., *Plesiomonas*, *Ewingella, and Morganella* spp*.,* which can participate in important infectious processes in humans [6]. The plasticity of the *E. coli* genome allows it to evolve, so that, in addition to existing as part of the intestinal biota, it allows it to settle outside the intestine. It has been shown that due to anatomical proximity, some enterobacteria that act as uropathogens, such as *E. coli,* may be associated with cases of BV and aerobic vaginitis. Moreover, the events of horizontal gene transfer between different species and genera allow the acquisition of new characteristics that benefit the host. Most of the emergence of bacteria resistant to antibiotics has been identified in this family of bacteria, and even recognized as causative agents of epidemics [7]. The proportion of strains resistant to antibiotics, including those that produce extended-spectrum lactamases, has increased exponentially [8,9], so that, almost all isolates of nosocomial origin and strains of the community are resistant to several classes of antimicrobials [10]. Among the mechanisms responsible for the acquisition of antimicrobial resistance, are mutation and gene acquisition by horizontal transfer mediated by plasmids, transposons, and recently, the integrons [11,12]. The integrons have been considered as natural expression cloning systems, with the ability to incorporate open reading frames through site-specific recombination and convert them into functional genes. Integrons can be classified into three classes [1-3], based on the sequence of the integrase gene, of which it is known that class 1 integrons are the most frequently detected in strains of clinical origin, and are associated with resistance to multiple antibiotics. More than 50 different class 1 integrons and 60 gene cassettes have been described, including gene cassettes that confer resistance to aminoglycosides, penicillins, cephalosporins, carbapenems, trimethoprim, chloramphenicol, rifampicin, erythromycin, and quaternary ammonium compounds [13-17]. Under this context, the objective of this work is to provide experimental evidence through the identification of the structural diversity of class 1 integrons, phylogenetic classification and serotyping, which allows to locate *E. coli* as possible primary pathogen causing AV, for the possible implementation of routine studies for the detection of *E. coli* causing vaginal infection. The implications and consequences of the identification of class 1 integrons and their resistance cassettes in virulent and commensal *E. coli* strains of vaginal origin are discussed.

Methodology

Ethical considerations

The institutional Committee of Research, Ethics, and Biosafety from "Hospital Juárez de México" approved the protocol under the registration number *HJM0330/17-I* in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" (http://www.conbioetica.mexico.salud.gob.mx/descargas/pdf/normatividad/normatinacional/10._NAL._Reglamento_de_ Investigacion.pdf). An informed consent was obtained from each participant prior to their recruitment into the study, and a self-administered questionnaire was gathered from each participant. The questionnaire gathered information about demographic characteristics.

Strains isolation, identification, and antimicrobial susceptibility assay

Bacterial strains belonging to the *Enterobacteriaceae* family were isolated from female patients with vaginal infections. Women were diagnosed with bacterial vaginosis if they were presented with three or more of Amsel's clinical criteria, from the "Hospital Juárez de México", from May 2017 to June 2017. Three hundred and three samples of vaginal exudates were plated on selective Mac Conkey agar, Sabouraud, Chloramphenicol agar, and non-selective Columbia agar plus 5% sheep blood,

Chocolate-PolyvitapleX agar (bioMérieux, Lyon, France), they were incubated aerobically and with an atmosphere of 5% CO_2 at 37 ºC for 24–48 hours, respectively [18]. Only cultures with an abundant development of colonies were included in the study. Typical colonies (lactose fermenting and non-lactose fermenting) were selected and purified on LB agar, and were subjected to presumptive identification by the Cowan and Steel criteria [19]. The identification of strains and the presumptive antimicrobial susceptibility were performed by using BD Phoenix™ (Brea, Californa, USA) automated identification and susceptibility testing system according to the manufacturer's protocol. The resistance phenotype of the strains was confirmed according to the criteria specified by the CLSI as follows.

Antimicrobial resistance confirmatory assay

The antimicrobial resistance to different antibiotics was confirmed by using the disk diffusion method on Mueller–Hinton agar plates according to the guidelines set by ''The Clinical and Laboratory Standards Institute" (CLSI 100-S21) [20]. The antimicrobial susceptibility was performed for twelve antimicrobial agents: amikacin (AN, 30 µg), ampicillin (AM, 10 µg), ampicillin/ sulbactam (SAM 10/10 µg), cefazolin (CZ, 30 µg), cefepime (FEP, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (AZ, 30 µg), ciprofloxacin (CIP, 5 µg), colistin (CL, 10 µg), ertapenem (ETP, 10 µg), fosfomycin (FOS, 200 µg), gentamicin (GM, 10 µg), imipenem (IPM, 10 µg), levofloxacin (LVX, 5 µg), meropenem (MEM, 10 µg), piperacillin/tazobactam (TZP, 10/100 µg), tigecycline (TGC, 100 µg), trimethoprim/sulfamethoxazole (SXT, 23.75/1.25 µg), and ceftriaxone (CRO, 30 µg) (BD, Brea, California, USA). *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 were used as controls. Results were inferred as susceptible or resistant by measuring the diameter of the inhibition zone according to the criteria specified by the CLSI (2018) [20].

Total DNA and plasmid isolation

Total DNA was extracted by using the Qiagen Mini kit (Qiagen, Courtaboeuf, France), and plasmid DNA from the strains was purified by using the PureLink™ Quick MiniPrep Kit (Invitrogen, Mannheim, Germany), both according to the manufacturer's protocol.

Detection of class 1 integrons and their gene cassettes

All isolates were screened for class 1 integron elements: integrase 5′(*int1I*)–variable region–(*qacEΔ1–sul1*)3′ by using the primers described in Table 1. The identity of resistance cassettes was also determined by sequencing the PCR amplicons. Sequencing was performed in the *"*Instituto de Biología, UNAM*"*, by using a DNA Analyzer 3730 ×l (Applied Biosystems, Foster City, California, USA) with the primers in–F or in–B (Table 1). Nucleic acid sequences were compared with the protein sequences online database (GenBank) by using the BlastX algorithm (http://blast.ncbi.nlm.nih.gov). *E. coli* carrying pAr–32 plasmid [IncU, class 1 integron (*intI1–aadA2–qacEΔ1/sul1*) In6 (*catA2*)], and *Aeromonas salmonicida* 718 carrying pRAS1 plasmid [IncU, class 1 integron (*intI1– dfrA16–qacEΔ1/sul1*), and Tn1721 (*TetA*)] were used as positive controls, and *E. coli* J53–1 (F− met− pro−) as negative control.

Table 1. Primers used in this study.

Primer	Molecular target	Sequence (5′ → 3′)	Size (bp)
IntI1-F	5′-Conserved Segment "Integrase *intI1*"	GTTCGGTCAAGGTTCTG	923
IntI1-R		GCCAACTTTCAGCACATG	
in-F	Variable region	GGCATCCAAGCAGCAAGC	From 150 to variable
in-B		AAGCAGACTTGACCTGAT	
qacEΔ1-F	3′-Conserved Segment "qacΔE1/sul11"	ATCGCAATAGTTGGCGAAGT	800
sul1-B		GCAAGGCGGAAACCCGCGCC	
ERIC1R	Intergenic consensus	ATGTAAGCTCCTGGGGATTCA	Variable
ERIC2		AAGTAAGTGACTGGGGTGAGC	
chuA-F	Hemin uptake system	TGCCGCCAGTACCAAAGACA	279
chuA-R		GACGAACCAACGGTCAGGAT	
yjaA-F	Unknown	TGAAGTGTCAGGAGACGCTG	211
yjaA-R		ATGGAGAATGCGTTCCTCAAC	
TSPE4.C2-F	Anonymous DNA fragment	GAGTAATGTCGGGGCATTCA	152
TSPE4.C2-R		CGCGCCAACAAAGTATTACG	
arpA-F	Ankyrin-like regulatory protein	AACGCTATTCGCCAGCTTGC	400
arpA-R		TCTCCCCATACCGTACGCTA	

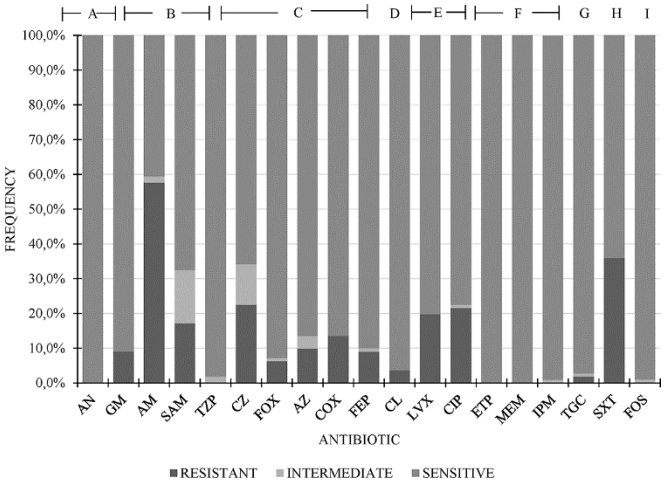
Molecular typing of strains carrying identical genetic arrays

Strains carrying identical genetic arrays (gene cassettes) in class 1 integrons, were analyzed by ERIC–PCR to differentiate between unique strains and clones. ERIC–PCR assays were performed to genotype the strains by using the ERIC1R and ERIC2 primers (Table 1). Amplification conditions were as follows: pre–denaturation at 95 °C for 7 minutes, denaturation at 90 °C for 30 seconds, annealing at 58 °C for 1 minute, and extension at 65 °C for 8 minutes (30 cycles), with a final extension at 68 °C for 16 minutes. Genetic pattern profiles were analyzed visually by intra–gel pattern comparison; a binary matrix was performed by using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). A dendogram was generated by using the Past software version 3.21, and Dice similarity index.

Bacterial phylogenetic assignment

The phylogenetic assignment was performed by using the protocol described by Clermont *et al.,* 2013 [21]. Primers for PCR amplification assays are shown in Table 1. PCR quadruplex conditions for gene fragments were performed as follows: 4 minutes at 94°C followed by up to 30 cycles of 94°C for 5 seconds, 59°C for 20 seconds, 72°C for 1 minute, and finally, 72°C for 5 minutes. *E. coli* LMM36-ULA (*chuA*+, *yjaA*+), and LMM32-ULA (*TSPE4.C2*+) strains were used as positive controls.

Figure 1. Antimicrobial resistance profile of *Escherichia coli* strains isolated from Mexican patients with vaginal infection. Antibiotics tested: Amikacin (AN), Gentamicin (GM), Ampicillin (AM), Ampicillin/Sulbactam (SAM), Piperacillin/Tazobactam (TZP), Cefazolin (CZ), Cefoxitin (FOX), Ceftazidime (AZ), Ceftriaxone (CRO), Cefepime (FEP), Colistin (CL), Levofloxacin (LVX), Ciprofloxacin (CIP), Ertapenem (ETP), Meropenem (MEM), Imipenem (IPM), Tigecycline (TGC), Trimethoprim/Sulfamethoxazole (SXT), and fosfomycin (FOS).



A) Aminoglycosides; B) ß-lactamics; C) Cephalosporins; D) Polymyxins; E) Quinolones; F) Carbapenemics; G) Glycylcyclines; H) Diaminopyrimidines; I) Phosphonates.

E. coli serotyping

The antigenic characterization was carried out based on the description by Orskov *et al*., 1975 [22], using two panels constituted by 186 sera prepared in rabbit against the somatic antigen (O), and 53 against the flagellar antigen (H) (SERUNAM Mexico, Mexico City). The agglutination reactions were performed in triplicate in 96-well round bottom microplates. To define the serotype of the strains, we considered the antigenic formula O: H obtained after evaluating the agglutination titers of the sera used.

Results

Description of the study population and bacterial isolation

A total of 303 Mexican patients with typical symptoms of vaginal infection (itching, burning during urination, abnormal vaginal discharge) were subjected to Amsel criteria in order to determine a possible bacterial infection. Additionally, microbiological isolation of strains belonging to the *Enterobacteriaceae* family was performed. Of the 303 samples analyzed, 262 were positive for bacterial infection; the above represents 86.5% of the samples. Of the samples positive for vaginal infection, 111 (42.36%) presented abundant development of typical colonies of bacteria belonging to the *Enterobacteriaceae* family.

Isolates identification

The automated identification showed that *Escherichia coli* (92 of 111 strains) was the main isolated microorganism (82.9%), followed by *Klebsiella pneumoniae* (*n* = 8/7.2%), *K. oxytoca* (*n* = 3/2.7%), *Enterobacter cloacae* (*n* = 2/1.8%), *Proteus mirabilis* (*n* = 2/1.8%), *Morganella morganii* (*n* = 1/0.9%), *Citrobacter youngae* (*n* =1/0.9%), *K. ozaenae* (*n* = 1/0.9%), and *Enterobacter aerogenes* (*n* = 1/0.9%).

Antimicrobial resistance assay

Nineteen different antimicrobials belonging to 9 families of antibiotics (aminoglycosides, ß- lactams, cephalosporins, polymyxins, quinolones, carbapenems, glycylcyclines, diaminopyrimidines, and phosponates) were tested in the 111 strains. Results showed that aminoglycosides, ß-lactams (piperacillin/tazobactam), cephalosporins, polymyxins, carbapenemics, glycylcyclines, and phosphonates were the

antimicrobials with the best antimicrobial activity against all strains tested. Penicillins, such as ampicillin, most of the cephalosporins, quinolones, and inhibitors of folate (diaminopyrimidines) showed lower inhibitory activity on the tested strains. Resistance and sensitive patterns for all strains are shown in Figure 1.

Detection of class 1 integrons and their gene cassettes

Class 1 integrons detection was performed under amplification strategy of conserved genes as follows: a first PCR reaction was performed to amplify the integrase ''*intI1*'' (5'CS). Once a positive amplification to first molecular target (923 bp), a second reaction to amplify *''qacEΔ1–Sul1''* (3'CS) was performed (800 bp). Finally, a third reaction was performed to amplify the variable region (flanked by conserved regions (*attI1* and *qacEΔ1*). Thirty complete integrons (*intI1*/variable region/*qacEΔ1–sul1)* were identified (27%). Interestingly, all strains carrying class 1 integrons were *E. coli.* The variable region amplicons ranged from 464 to 1610 bp, indicating different genetic arrangements into class 1 integrons. The criteria for defining the identity of the arrangements obtained were percentage match (usually > 75 %), length of match (usually > 100 bp), and probability of similarity. The sequences showed inserted cassettes corresponding to genes coding for adenylyltransferases (*aadA1, aadA2, aadA5,* and *aadA22*), dihydrofolate reductases (*dfrA1, dfrA5, dfrA5, dfrA17*), and oxacillinase (bla_{OXA-1}). Ten different arrays (A to J array) were identified in the 30 strains of *E. coli*. Array genetic "I" (*dfrA17-aadA5)* was predominantly prevalent over other genetic arrays identified in the study (16/30). This genetic arrangement has the same type of cassettes than the arrangement found in the 1058 strain, but in different orientation (F array). Single arrays with cassette variants *dfrA* (A array), and *aadA* (B, C, and D arrays) were identified in 4 different strains (0024, 0056, 1106,

Figure 2. Schematic map of class 1 integrons identified in *Escherichia coli* strains isolated from patients with vaginal infection and associated resistance phenotype. Gene cassettes (not in scale) are shown as boxes, the arrows indicate the orientation of the transcription. The 5´and 3 ´conserved segments are annotated. Letters A–J indicate the arrangements found.

Array	5´CS	3´CS	Variable region (bp)	Strains (ID)	Associated resistance phenotype	ID sequence GeneBank**
A	*intI1*	*dfrA5* – *qacEΔ1* – *Sul1*	464	0024	Trimethropim and sulfamethoxazole	HQ880248.1
B	*intI1*	*aadA1* – *qacEΔ1* – *Sul1*	779	0056	Streptomycin and sulfamethoxazole	KU956957.1
C	*intI1*	*aadA2* – *qacEΔ1* – *Sul1*	791	1106	Streptomycin and sulfamethoxazole	KJ363320.1
D	*intI1*	*aadA22* – *qacEΔ1* – *Sul1*	779	4074	Streptomycin and sulfamethoxazole	KU956958.1
E	*intI1*	*dfrA12* – *aadA2* – *qacEΔ1* – *Sul1*	1238	5120, 003A, 3349, 5123	Trimethropim, streptomycin and sulfamethoxazole	KR734342.1
F	*intI1*	*aadA5* – *dfrA17* – *qacEΔ1* – *Sul1*	1261	1058	Streptomycin, trimethropim and sulfamethoxazole	KT175895.1
G	*intI1*	*dfrA1* – *aadA1* – *qacEΔ1* – *Sul1*	1252	2103, 2393	Trimethropim, streptomycin and sulfamethoxazole	KT305946.1
H	*intI1*	*dfrA17* – *aadA2* – *qacEΔ1* – *Sul1*	1238	0030	Trimethropim, streptomycin and sulfamethoxazole	KT175895.1 and KJ363320.1
I	*intI1*	*dfrA17* – *aadA5* – *qacEΔ1* – *Sul1*	1261	*	Trimethropim, streptomycin and sulfamethoxazole	KT175894.1
J	*intI1*	bla_{OXA-1} – *aadA1* – *qacEΔ1* – *Sul1*	1610	1148, 2063	Azlocillin, streptomycin and sulfamethoxazole	JN003856.1

* 1071, 1116, 5142, 5145, 7180, 7104, 0036, 3255, 0026, 2082, 6096, 3088, 0047, 0361, 0074, 4362; **Sequence producing significant alignment with sequence identified in this work.

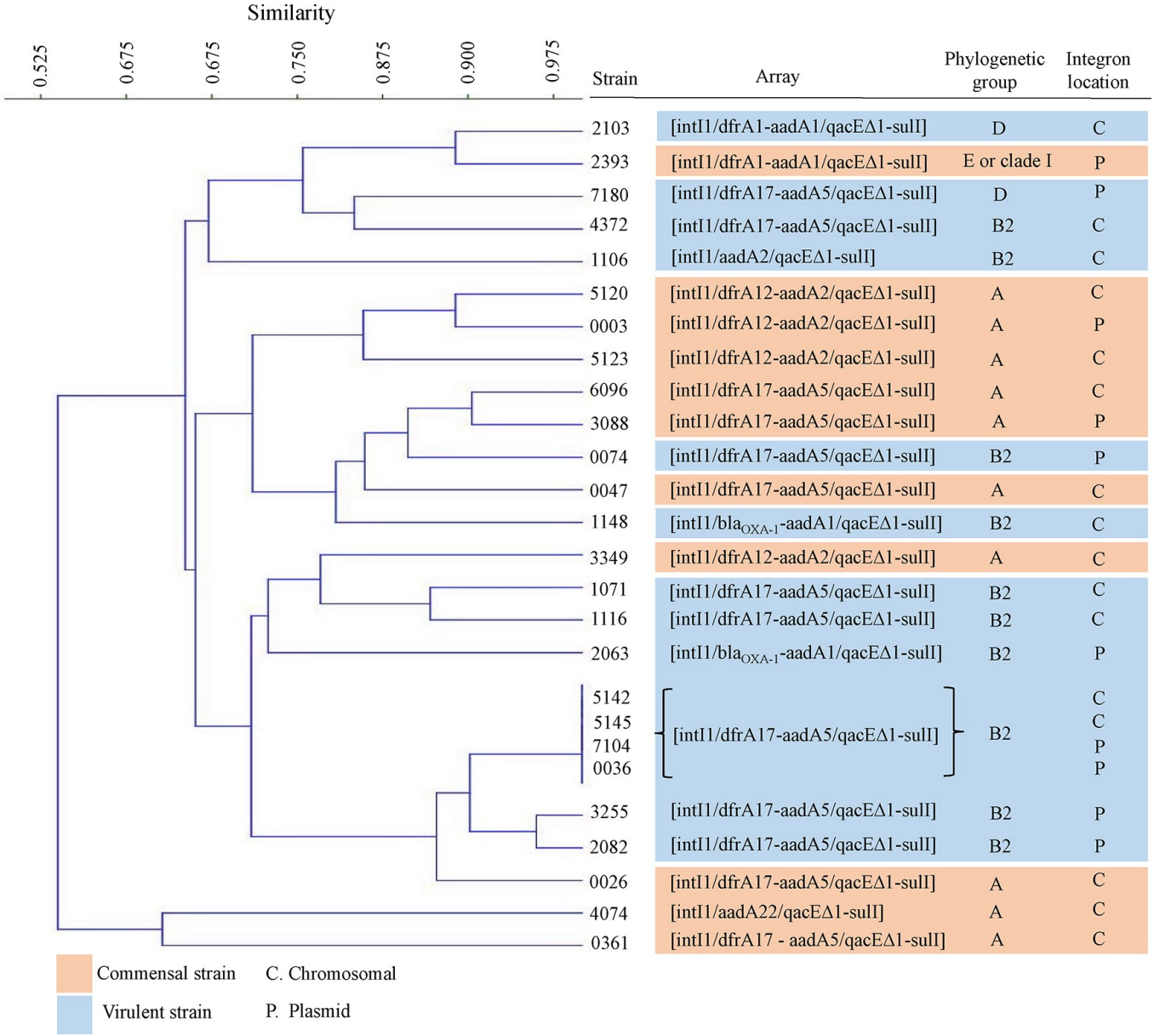
and 4074 strains). Three different combinations of unique genetic arrangements conformed by two variants of *dfrA* and *aadA* cassettes were identified in 7 different strains (E, G, and H arrays).

Finally, two strains showed an array with cassettes bla_{OXA-1} and *aadA1* (array J) (Figure 2). In order to define the location of integrons (chromosome or plasmid), both, plasmid and total DNA were purified and used as templates for integron elements amplification (only variable region). Only seventeen of the 30 strains showed integrons located in plasmids.

Molecular typing of strains carrying identical genetic cassettes arrays

The clonal relationship between strains carrying identical genetic cassettes arrays in class 1 integrons (n = 26 genetic arrays) was analyzed by using enterobacterial repetitive intergenic consensus (ERIC-PCR). The electrophoretic analysis of PCR reaction products (amplicons) of the evaluated strains revealed that the number of bands ranged from 10 to 16 in different profiles. The sizes of the amplicons ranged from slightly more than ≈ 150 bp to about ≈ 1800 bp. Products in the range of 200–400 bp were more frequently found. The diversity in intergenic regions

Figure 3. Clonal distribution obtained by ERIC-PCR assays between strains of *Escherichia coli* carriers of class 1 integrons with identical arrays and their phylogenetic relationship by the Clermont algorithm



identification allowed the differentiation of 26 strains, which were clustered in 23 different genetic groups. This probe showed that 22 of 26 *E. coli* strains are unique and not clones, as the profiles obtained were different from each other. Only 4 strains (5142, 5145, 7104, 0036) were indistinguishable from each other (100% similarity); interestingly, these strains carried the same genetic array (*dfrA17-aadA5*). Clonal distribution obtained by ERIC-PCR between strains of *E. coli* strains carriers of class 1 integrons and their phylogenetic relationship by Clermont algorithm is shown in Figure 3.

Bacterial phylogenetic assignment

All strains of *E. coli* (with or without integron) (n = 92) were subjected to genotyping classification according to the Clermont algorithm. The phylogenetic group A (43/46.7%) was the most predominant, followed by group B2 (40/43.5%), group D (7/7.6%), and finally group E or clade I (2/2.2%). The phylogenetic distribution of the integron carrier strains (n = 30) was: group A (14/15.2%) being the most predominant, followed by group B2 (13/14.1%), group D (2/2.2), and group E or clade I (2/1.1%).

Somatic and flagellar serotyping

Serological typing allowed the identification of 52 different serotypes. The analysis showed the presence of serotypes O25: H4, O101: H-, O1: H47, and O2: H6 corresponding to strains of uropathogenic *E. coli* (UPEC), serotypes such as O6:H1, OR: H-, O165: H -, and O15: H- corresponding to strains of Shiga toxin.-producing *E. coli* (STEC). Serotypes O55: H2, O55: H21, and O18ab: H14 (1.1%) corresponding to strains of enteropathogenic *E. coli* (EPEC). Finally, serotypes O129: H27 and O104: H4, which belong to enterohemorrhagic *E. coli* (EHEC) and O129: H34, and O129: H4 belonging to enteroinvasive *E. coli* strains (EIEC).

Discussion

To our knowledge, until now, there is no work describing the structural diversity of antibiotic resistance cassettes in class 1 integrons carried in virulent and commensal strains of *E. coli* isolated from patients with vaginal infection. In the present study, a high frequency of isolation of strains belonging to the family *Enterobacteriaceae* was identified in the analyzed population. Even when the genus of this bacterial family is a natural inhabitant of the intestinal tract, they may be involved in intestinal, genitourinary, blood infections, among others [23]. Under this antecedent, and since *E. coli* was the most isolated bacterial pathogen, genotyping (classification in commensals and virulent) of the strains of *E. coli* was carried out. The high prevalence of vaginal infection associated with *E. coli* in symptomatic patients (35.11%) identified in the study, exceeds the incidence reported by Donders *et al.,* 2017 [24]. In this work, *E. coli* has been recognized as a causative agent of aerobic vaginitis, with a prevalence of up to 23% among symptomatic women. This type of vaginal infection is characterized by colonization of the mucosa causing an intense inflammatory response [4]. In addition, other of the microorganisms identified were *K. pneumoniae, K. ozaenae, K. oxytoca, Enterobacter cloacae, E. aerogenes, Citrobacter youngae,* and *Proteus mirabilis.* These bacterial genera are considered of clinical importance in other anatomical regions of the body; however, in the diagnostic laboratories they are not routinely identified as causative agents of aerobical vaginitis [6,24]. Even when they are not routinely identified, there are studies where *K. pneumoniae* is related as an opportunistic pathogen in cases of vaginitis [25], and *Enterobacter* spp. in cases of vaginal infection [26]. The importance in the identification of the causative agents and the profile of antimicrobial resistance in this type of infections, has the objective of providing an appropriate treatment, as it may cause persistent infection, pelvic inflammatory disease, infertility, ectopic pregnancy, reproductive dysfunction, increased risk of postoperative infection, predisposition to HIV acquisition, and premature birth [27]. Regarding the resistance profiles, it was observed that the strains showed greater resistance to the ß-lactams such as ampicillin (59.5%), and ampicillin-sulbactam (32.4%). This result is coincident with that reported by Repessé *et al.,* 2017 [10], where it is described that *E. coli*, *K. pneumoniae,* and *E. cloacae* are intrinsically resistant to β-lactams. The antibiotics with the best antimicrobial activity (100%) were some aminoglycosides and carbapenems. These antibiotics are used in the case of the appearance of multiresistant strains to most of the antibiotics of first choice. Even though a small number (3.6%) of colistin-resistant strains were identified, their detection is of epidemiological interest. For colistin, CLSI and EUCAST recommended the technique of broth microdilution as the only method to assess the sensitivity to this antibiotic in Enterobacteria, *P. aeruginosa,* and *Acinetobacter* spp, due to the high error rates. However, in Mexico this methodology is not performed systematically in diagnostic laboratories. There is a recommendation of EUCAST disk diffusion

technique, the cut-off points defined by the Latin American Network of Regional Reference Laboratory for Antimicrobial Resistance Surveillance (R ≤ 16 mm, I = 17-20 mm, S ≥ 21 mm) [28]. Future work is aimed at the identification of the *mcr* gene and its variants, responsible for resistance to this antibiotic [29]. Currently, colistin is considered the antibiotic of last resort in the treatment of infections by Gram-negative bacteria, such as *K. pneumoniae* and *E. coli* [30]. In the case of gentamicin and ciprofloxacin, resistances of 9.0% and 22.5%, respectively were identified; with the data reported by Diaz *et al.,* 2004 [31], where frequencies of 65 and 30% were reported for these antibiotics in *K. pneumoniae* isolates of clinical origin. Kaushik *et al.,* 2017 [23] describe that class 1 integrons have been identified in collections of *E. coli* isolated since 1973, from locations that represent almost all parts of the world, and that the emergence of these genetic structures is due to an adaptive mechanism to the irrational and indiscriminate use of antibiotics. The integrons are recognized as the systems responsible for the dissemination of resistance genes through the capture, storage, and rearrangement of cassettes that encode resistance to antibiotics [13]. Class 1 integrons constitute the majority of integrons found in isolates of clinical origin, and are associated with the resistance to antibiotics. To investigate the identity of the genetic arrangements within the class 1 integrons in *E. coli* strains, we performed a BLAST analysis, by using strict filter parameters with more than 99% of nucleotide identity, and at least 80% query coverage. The genetic cassette arrays identified in class 1 integrons correspond to variants of resistance cassettes to trimethoprim (*dfrA*), streptomycin, spectinomycin (*aadA*), azocillin, piperacillin, and mezlocillin (bla_{OXA}). The resistance phenotype associated with the genetic arrangements identified in the integron carrier strains was confirmed. A high prevalence of polygenic arrangements was identified (*n* = 26/86.7) in comparison with monogenic arrays that were only identified in 4 strains (13.3%). The arrangement with the highest prevalence was df*rA17-aadA5* (*n* = 16/53.3%). An arrangement consisting of the same genes in the strain 1058 was identified, but in reverse order "*aadA5-dfrA17"* (3.3%). This arrangement has been more frequently described in Gram-negative bacteria of the genera *Salmonella* spp., and *Escherichia* spp. [14,15]. It was originally identified in several isolates in the early 90's. It is believed that its appearance was due to the irrational use of antibiotics from the family of aminoglycosides and diamonipyrimidines [16]. This observation is similar to the second most frequently identified polygenic arrangement (*n* = 4/13.33) corresponding to *dfrA12-aadA2*. Polygenic arrangements with cassette variants of *dfrA* and *aadA* were less frequently identified in this work (6.66%); the "*drfA1-aadA1*" arrangement has been identified in other works with low frequencies (≈ 5%) in strains of human origin and food, and strains that have been carried in conjugative plasmids [17]. To our knowledge, this is the first work describing the *dfrA17-aadA2* arrangement identified in an isolate of human origin; even when it was in low prevalence (3.33%). Previously, this arrangement has only been identified in extra-intestinal pathogenic *E. coli* isolates from swine in China [32]. Four single monogenic arrays with cassettes *dfrA5*, *aadA1, aadA2,* and *aadA22* were identified at a lower frequency (3.33% each). Experimental evidence on the appearance of integrons with monogenic arrays suggests that *aadA1* and *aadA2* type cassettes possibly appeared in the 80's and 90's, whereas monogenic integrons with *dfrA5* cassettes emerged recently (90's and 2000's) [16]. Regarding the *aadA22* monogenic arrangement, this has previously been described in strains of *E. coli* of commensal origin [33], which suggests that commensal strains that are theoretically innocuous to humans are capable of capturing extra chromosomal genetic material, and which could provide selective advantages to adapt to adverse environments. Due to the fact that some strains presented identical genetic arrangements, the genetic relationship between them was studied by ERIC-PCR assays. It is known that clonal expansion also contributes to the current high prevalence of interregional propagation of bacterial species carrying integrons [34]. The results showed a wide genetic diversity in most strains. Moreover, there are some strains (*n* = 4) with clonal behavior. Some evolution models propose that the diversity in pathogenicity is due to the acquisition of virulence genes. *E. coli* is the result of constant acquisition of these. Therefore, with the results obtained we decided to carry out a phylogenetic analysis to determine the virulence of the strains under study. Clermont *et al*., in 2013 proposed a dichotomous analysis based on the presence of the *chuA, yjaA* genes and the anonymous fragment designated as TspE4.C2 proposing 4 main phylogenetic groups, virulent extraintestinal strains belong mainly to groups B2 and D, while the commensal strains belong to group A and B1. In addition, the additional gene *arpA* was added, which makes the genotyping method a quadruplex PCR assay [21]. Under this antecedent, the phylogenetic classification of all strains of *E. coli* was performed by using the algorithm reported by Clermont

et al., 2013. Although about 50% of the strains were classified in the non-virulent phylogenetic groups, an important proportion of these strains (n = 14) presented class 1 integrons. A previous work has shown the presence of these genetic structures in strains of *E. coli* of commensal origin [35]. Due to the detection of these elements of antimicrobial resistance in commensal strains, we can speculate that these strains participated in horizontal DNA transfer events. Since *E. coli* has great genetic diversity, it is considered a bacterium with a high genomic plasticity, including loss and gain of genetic material [36]. Therefore, the acquisition of new cassettes in integrons could allow commensal bacteria to cause persistence and possible infection, due to the acquisition of new genetic material that evades the antimicrobial activity. Clermont *et al.,* 2013 [21] reported that commensal strains with a moderate resistance profile and low virulent potential can remain in the epithelium, and can cause infections in immunosuppressed patients. Serological typing (somatic and flagellar) plays an important role in the clinical diagnosis of *E. coli* [22]. The typing was performed, with the aim of knowing the serological distribution in the strains studied. The results revealed the presence of serotypes of UPEC strains, the predominant agent of urinary tract infections. UPEC expresses adhesins, flagella, iron uptake systems, and exopolysaccharides to colonize the urinary tract [37]. Serotypes of STEC strains were also identified; this bacterial group has been associated with diarrhea, hemorrhagic colitis, and haemolytic uremic syndrome [38]. STEC is considered zoonotic with ruminants, particularly in sheep and cattle [39]. Evidence shows that birds, dogs, horses, and pigs are also reservoirs of STEC, indicating that there are other sources of infection than ruminants [40]. Furthermore, serotypes corresponding to EPEC strains were detected, previously described as pathogens associated with diarrhea in children, and which produce a histopathology finding described as "adherence and sloughing" [41]. EIEC infection is commonly manifested as watery diarrhea, which may be indistinguishable from the secretory diarrhea observed with ETEC [42]. Only a minority of patients experience dysentery syndrome, which manifests as blood, mucus, leukocytes in feces, tenesmus, and fever [43]. These type of strains were identified in 2.2% of the strains analyzed. An unexpected result was the finding of a strain with serotype O104: H4. Previous works have reported the detection of outbreaks associated with this serotype which spread throughout Germany and later in France [44]. This new EHEC clone generated a total of 46 deaths, 782 cases of haemolytic uremic syndrome, and 3,128 cases of acute gastroenteritis. The majority of patients (although diagnosed in different countries of Europe) became infected in Germany or France. This outbreak was caused by an EHEC strain similar to enteroaggregative *E. coli* (EAEC) of serotype O104: H4. However, an important difference is the presence of a prophage encoding the Shiga toxin (Stx), which is characteristic of the EHEC strains [45], this fact makes it strange to detect this serotype in a strain of vaginal origin.

Conclusion

The results in this study demonstrate the presence of *E. coli* (virulent and commensal) of vaginal origin carrying class 1 integrons. These strains represent an important reservoir of resistance genes, with the possibility of capturing new resistance cassettes. These evidences should serve as guidance for the modification of protocols in the diagnosis of aerobic vaginitis as well as the development of policies for the rational use of antimicrobials in the therapy of vaginal infections.

Acknowledgements

M.B.L. received grant-aided support from "Consejo Nacional de Ciencia y Tecnología" (CONACyT, México), while G.C.E, J.M.B.L., and G.I.C received support from the "Sistema Nacional de Investigadores (SNI)" from CONACyT, Mexico. G.C.E. received support from Estímulos al Desempeño en Investigación, Comisión y Fomento de Actividades Académicas (Instituto Politécnico Nacional). This study was funded by Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (SIP 20180060 and 20194936). The authors are also grateful to *Sofia Mulia* for correcting the style of the manuscript.

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Conflict of interests: No conflict of interests is declared.