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Original Article

O-serogroups of multi-drug resistant cervicovaginal *Escherichia coli* harboring a battery of virulence genes☆

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ABSTRACT

Multi-drug resistant cervicovaginal *Escherichia coli* (CVEC) infections are a serious health problem. The aim of this study is to determine the patterns of virulence genes, antibiotic resistance and O-serogroups of CVEC isolated in Mexico. Two hundred strains of CVEC were isolated from women attending two Clinics at the Instituto Mexicano del Seguro Social. *E. coli* O-serogroups and virulence markers were identified by PCR. Antibiotic susceptibility was determined using the Kirby-Bauer disc-diffusion method. Serogroups O25 (50%), O75 (9%) and O15 (7.5%) were the most frequent among the CVEC strains isolated. The frequencies for antibiotic resistance were ampicillin 97%, (n = 194); carbenicillin 93.5%, (n = 187); cefalotin 77%, (n = 154); and nitrofurantoin 71%, (n = 142). The frequency of multiresistant isolates (3–12 drugs) was 197 (98.5%). The most frequent virulence genes found were *feoB* (91.5%), *fimH* (89.5%), *kpsMT11* (75%), *iutA* (66%), and *iroN* (59%). One hundred and four distinct patterns of virulence markers with antibiotic-resistance genes associated with O-serogroups were identified amongst CVEC isolates. In conclusion: most CVEC strains isolated were multiresistant to antibiotics, belonged to three O-serogroups, and possessed a battery of virulence factors. This knowledge may lead to improved guidelines and standards for treating cervicovaginal infections.

1. Introduction

Vaginal infections are one of the most common and recurrent diseases affecting women, particularly those of reproductive age [1]. One of the bacterial species that is responsible for vaginal infections is cervicovaginal *Escherichia coli* (CVEC) [2]. The acuteness or chronicity of cervicovaginal infections caused by CVEC is related to a large number of virulence factors, including adhesins, iron acquisition systems (nutrition), protectins, and toxins [3]. Many of these virulence genes are horizontally transferred between bacteria via pathogenicity-associated islands (PAIs) [4]. CVEC strains have been associated with certain specific O-serogroups [5].

The multiresistance of *E. coli* strains to several groups of antibiotics represents a severe health problem that reduces the therapeutic options for treating infections [6]. Because of the significant social and economic burden of vaginal infections, together with the need for the molecular characterization of CVEC strains, the objective of this study is to determine the association pattern of virulence genes, antibiotic resistance, and O-serogroups of CVEC strains isolated in Mexico.

2. Materials and methods

2.1. Patient selection and sampling

We studied 210 women aged 18–69 years with signs and symptoms of cervicovaginal infection from the outpatient

☆ All authors meet the ICMJE authorships criteria.

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department of gynaecology of the Family Medical Units no. 62 and 64 at the Instituto Mexicano del Seguro Social (IMSS). The IMSS Ethics Committee and the IMSS National Research Commission approved the study (Registry number R-2017-785-032). The selected patients reported no treatment with antibiotics in the past 3 months. All the patients signed an informed consent form. The *E. coli* strains were identified by standard microbiological methods and amplification of the 16S rRNA gene by PCR as has been described by Momtaz et al. [7] and using the *E. coli* strain ATCC 11775 as a control.

2.2. Susceptibility to antibiotics

The Kirby-Bauer disc-diffusion assay (Bio-Rad, Mexico) was used to determine the susceptibility to 12 different antibiotics (ampicillin, carbenicillin, cephalothin, nitrofurantoin, trimethoprim & sulfamethoxazole, norfloxacin, ciprofloxacin, netilmicin, gentamicin, cefotaxime, chloramphenicol, and amikacin). The *E. coli* strain ATCC 25922 was used as a control for the reproducibility of this method. The data were interpreted according to Clinical and Laboratory Standards Institute guidelines [8].

2.3. Identification of O serogroups

O-serogroups were identified by multiplex PCR as was described by Li et al., [9]. Pairs of primers used targeted the genes *wzx* of O1, O4, O7, O16, O18, O21, O22 and O83 serogroups, *wzy* of O2, O6, O15, O25 and O75, and *orf486* of O8. The 14 pairs of primers were divided into two groups for multiplex PCR; in the first group the serogroups identified were O1, O6, O7, O8, O16, 021 and O75, and in the second group the serogroups identified were O2, O4, O15, O18, O22, O25 and O83.

2.4. Detection of virulence genes

The genes *fimH* (type-1 fimbriae), *papA* (fimbrial major pilin protein), *papEF* (fimbrial protein), *focG* (F1C fimbriae), *iss* (increased serum survival protein), *kpsMT11* (K-antigen), *iuc* (aerobactin), *iroN* (iron), *iutA* (ferric aerobactin receptor), *feoB* (ferrous iron transport protein B), *hlyA* (haemolysin), *cnf1* (cytotoxic necrotising factor 1), *set-1* (Shigella enterotoxin 1), *astA* (enteroaggregative heat-stable toxin), and PAIs-related gene *malX* were detected as previously described [7,10].

3. Results

In this study, *E. coli* was identified by standard microbiological methods and PCR in 95.2% (n = 200) of vaginal cultures. Most CVEC strains were resistant to beta-lactam antibiotics (ampicillin, carbenicillin, and cephalothin) (Table 1). Most strains (n = 197) were multiresistant to antibiotics, and 79% of the strains (n = 158) were multiresistant to three to nine antibiotics (Table 1). Only three strains (1.5%) were resistant to only two antibiotics.

In addition, 79.5% (n = 159) of the strains were assigned to nine of the fourteen serogroups, the most common being O25 (n = 100), O75 (n = 18), and O15 (n = 15) (Table 2).

The adhesion gene *fimH* was the most frequently identified, mainly associated with serogroups O25, O75, and O15, whereas protectin gene *kpsMT11* was most commonly associated with serogroups O25 and O75 (Table 3).

The genes *feoB*, *iutA*, and *iroN*, which encode iron acquisition systems (nutrition), and the toxin gene *cnf1,* were most frequently detected in serogroups O25, O75, and O15 (Table 3). The PAIs-associated gene *malX* was more common in strains from serogroup O25.

Table 1
Multi-resistance to antibiotics in the CVEC strains.

Antibiotics		No. of resistant strains (%)
Ampicillin		194 (97)
Carbenicillin		187 (93.5)
Cephalotin		154 (77)
Nitrofurantoin		142 (71)
Trimethoprim & sulfamethoxazole		121 (60.5)
Norfloxacin		117 (58.5)
Ciprofloxacin		116 (58)
Netilmicin		83 (41.5)
Gentamicin		83 (41.5
Cefotaxime		76 (38)
Chloramphenicol		59 (29.5)
Amikacin		45 (22.5)
Multi-resistance	3–5	63 (31.5)
No. of antibiotics	6–9	95 (47.5)
	10–12	39 (19.5)

4. Discussion

Bacterial vaginosis is one of the most common causes of vaginal pain in women of reproductive age [11]. Bacterial vaginosis is caused by disequilibrium of the vaginal microbiota characterized by the absence of Lactobacilli and the presence of non-virulent bacteria like Gardnerella and other anaerobic bacteria. Bacterial vaginitis is a disease causing a lot of purulent discharge, vulvar pain and burning feeling. Vaginosis should be distinguished from vaginitis, since it is not recommended to treat the former intensively with antibiotics, which may eliminate some beneficial bacteria, like Lactobacilli, residing in vagina of healthy women. Rates of bacterial vaginosis can reach 51% in this group [12] and vary from 23% to 38% in postmenopausal women [13]. *E. coli* is reported as the most common bacterial species involved in cervicovaginal infections [14]. Our results confirm this observation as we identified *E. coli* in 95.2% (n = 200) of vaginal samples. To elucidate the virulence potential of *E. coli* strains involved in vaginal infections, we analysed the different association patterns of virulence factors and antibiotic resistance in CVEC strains from different O-serogroups.

The O-serogroups most commonly associated with cervicovaginal infections were O25, O75, and O15 (Table 2). These results are similar to those previously reported for CVEC strains, wherein the most common serogroups were O25, O15, and O6 [3]. The frequency of O-serogroups in CVEC strains in this study is in agreement with other studies involving uropathogenic *E. coli* (UPEC) strains [7,15].

Table 2
O-serogroups identified in the CVEC strains.

Serogroup	Number (%)
O1	3 (1.5)
O2	0 (0)
O4	0 (0)
O6	5 (2.5)
O7	0 (0)
O8	5 (2.5)
O15	15 (7.5)
O16	7 (3.5)
O18	3 (1.5)
O21	3 (1.5)
O22	0 (0)
O25	100 (50)
O75	18 (9)
O83	0 (0)
Non-detected	41 (20.5)
Total	**200 (100)**

Table 3
Virulence genes associated with the O-segroups in the CVEC strains.

Gene		Serogroup (No.)														
		01 (3)	02 (0)	04 (0)	06 (5)	07 (0)	08 (5)	015 (15)	016 (7)	018 (3)	021 (3)	022 (0)	025 (100)	075 (18)	083 (0)	Nontypeable (41)	No. (%)200 (100)
Adhesins	*fimH*	2	–	–	5	–	5	15	3	3	2	–	95	17	–	32	179 (89.5)
	papA	–	–	–	2	–	–	–	–	1	–	–	22	1	–	2	28 (14)
	papEF	–	–	–	3	–	–	6	1	2	–	–	32	2	–	7	53 (26.5)
	focG	–	–	–	1	–	–	2	1	–	1	–	13	–	–	–	18 (9)
Protectins	*iss*	–	–	–	–	–	1	1	–	–	1	–	7	1	–	4	15 (7.5)
	kpsmT11	2	–	–	5	–	2	7	6	2	1	–	85	15	–	25	150 (75)
Nutrition	*iuc*	3	–	–	–	–	2	2	1	1	–	–	37	2	–	7	55 (27.5)
	iroN	1	–	–	4	–	5	9	7	2	1	–	62	7	–	20	118 (59)
	iutA	3	–	–	–	–	1	7	4	3	–	–	82	15	–	17	132 (66)
	feoB	3	–	–	5	–	4	15	7	3	2	–	92	18	–	34	183 (91.5)
Toxins	*hlyA*	–	–	–	3	–	1	2	–	1	1	–	35	2	–	1	46 (23)
	cnf1	–	–	–	3	–	2	4	1	–	1	–	48	2	–	5	66 (33)
	set1	–	–	–	4	–	1	–	1	1	–	–	13	1	–	2	23 (11.5)
	astA	–	–	–	–	–	–	1	–	–	–	–	5	–	–	–	6 (3)
PAI	*malX*	2	–	–	3	–	1	4	1	1	–	–	59	6	–	8	85 (42.5)

These results indicate that most CVEC strains (n = 197, Table 1) are multiresistant to three to 12 antimicrobial agents, particularly to beta-lactams (ampicillin, carbenicillin, and cephalothin), as well as nitrofurantoin, trimethoprim, sulfamethoxazole, and norfloxacin (Table 1). The high levels of antibiotic resistance corroborate with the prevalence of multiresistance among *E. coli* strains found in other parts of the world [16,17]. The high frequency of multiresistant CVEC strains found in this study could be due to that before 2010, antibiotics were sold freely in the pharmacies, without requiring that they were prescribed by a doctor, and also may be attributed to the extensive prescription of antibiotics in the public health sector in Mexico to manage genitourinary infections. This practices have allowed the selection of multiresistant strains capable of horizontally transferring antibiotic resistance genes to other strains via mobile genetic elements, including plasmids, transposons, and integrons [18]. Bacterial multiresistance to antibiotics is considered a severe health problem that reduces the number of treatment options for infections [19]. The resistance to ampicillin, cefotaxime, and amikacin detected in vaginal *E. coli* strains (Table 1) was higher than that in multiresistant UPEC strains described by Liu et al. [20], whereas resistance to gentamicin was lower in the strains evaluated in this study. The rate of multiresistance and individual resistance to ampicillin, carbenicillin, cephalothin, trimethoprim, sulfamethoxazole, and chloramphenicol in CVEC strains was similar to that previously reported by our group in UPEC strains from serogroups O25 and O15 isolated from patients with urinary tract infections in Mexico [15]. These findings suggest that there has been a notable increase in the prevalence of multiresistant genitourinary *E. coli* strains in Mexico. Multiresistance of vaginal bacteria to antibiotics poses a serious problem that has been faced up with intravaginal or oral treatments with probiotics that rise vaginal pH and with inhibitors or disruptors of bacterial biofilm formation, among others treatments [21]. Adhesins are considered the most important pathogenicity factor in *E. coli*. These molecules can trigger signalling pathways between bacteria and host cells and promote tissue colonization and invasion [22]. Our results indicate that the most common adhesion genes in CVEC strains were *fimH* and *papEF*, and these genes were primarily associated with serogroups O25, O75, and O15 (Table 3). The gen *fimH* is common in vaginal *E. coli* strains from serogroups O25, O15, and O6 [3], while the genotype *pap* is common in UPEC strains from serogroups O55 and O25 [7]. The presence of *fimH*, *papA*, and *papEF* in *E. coli* (Table 3) may increase the risk of severe infections because these markers have been associated with cystitis and pyelonephritis [23].

The marker *kpsMT11* was common in CVEC strains (Table 3), and this result agrees with the frequencies found in other CVEC [5] and UPEC strains [24]. The presence of *kpsMT11* in the evaluated strains may be related to the chronicity or recurrence of vaginal infections since the capsular K antigen has been shown to promote resistance to phagocytosis and avoidance of complement-mediated death [25].

Iron is necessary for several cellular processes, including electron transfer during cellular respiration [26], and favours bacterial survival and multiplication during the course of infection. The most frequent genes encoding iron acquisition systems (nutrition) in CVEC strains were *feoB*, *iutA*, and *iroN*, and were predominant in serogroups O25, O75, and O15 (Table 3). The frequency of *feoB* and *iutA* in CVEC strains were higher than that in *E. coli* strains isolated from urinary tract infections in children [24]. Similarly, *iroN* frequency in CVEC strains was higher than those found in uropathogenic strains of *E. coli* [27].

Toxin genes are important for bacterial colonization and inflammatory responses during infections. In this study, the most frequently identified toxin genes were *cnf1* and *hlyA*, and these genes were more common in serogroup O25 (Table 3). However, this frequency is lower than that previously found in vaginal *E. coli* strains [2] and strains isolated from patients with urinary tract infections [28]. The presence of the genes *cnf1* and *hlyA* in CVEC strains may increase the acuteness of infections, as previously observed in UPEC strains. Furthermore, *cnf1* and *hlyA* are important pathogenicity markers in strains that cause pyelonephritis [29]. In the next future we will address the prevalence of virulence genes and O-serotypes of CVEC strains with those of UPEC strains, because *E. coli* colonize the vagina before invade the urinary tract.

5. Conclusion

Most of the CVEC strains isolated are multiresistant to antibiotics, belong to three O-serogroups (O25, O75, and O15), and possess a battery of virulence factors. This knowledge provides valuable insight for the formulation of guidelines for treating cervicovaginal infections.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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