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Antimicrobial resistant *Escherichia coli* in the reproductive tract microbiota of cows and sows

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ABSTRACT

Escherichia coli is a natural colonizer of the urogenital mucosa of healthy females; however it is one of the pathogens associated to reproductive failures in cows and sows. A better knowledge about the characteristics of native *E. coli* will allow us to differentiate them from pathogenic strains. Ninety autochthonous isolates from the reproductive tract of sows and cows were characterized to determine the phylogenetic profile, antibiotic resistance and virulence factors; also, comparisons between different breeding systems were performed. Vaginal colonization of *E. coli* was statistically higher in cows (57.5%) than sows (23.8%), and most isolates belonged to the phylogenetic group A: 79.69 and 80.77%, respectively; moreover phylo-groups B1 (12.5 and 11.54%) and D (7.81 and 7.69%) were significantly lower; however, none was classified as B2. Positive associations between virulence factors and group D were found. Isolates with antimicrobial susceptibility were associated with group A and the MDR (Multiple Drug Resistance) was related to the porcine source. These results contribute to the knowledge of extra-intestinal *E. coli* populations; which could affect the reproductive performance of females.

1. Introduction

Escherichia coli has been associated with a wide range of diseases in farm animals that cause high economic losses due to mortality, morbidity and treatment costs, and also increase the risk of inducing an impairment in food quality and therefore in human health [1,2]. A special impact in the productivity is produced by extra-intestinal infections caused by *E. coli*, such as metritis in cattle [3], uterine and urinary tract infections in pigs [4,5]; these infections represent important causes of infertility in females in livestock [5,6]. However, *E. coli* was also described in the autochthonous bacterial communities of vagina from healthy cows [7]. Moreover, Otero et al., showed that *E. coli* is part of the vaginal microbiota in heifers from weaning to breeding [8]. Bara et al. [9] studied the microbial colonization of cervix and vagina of sows and found *E. coli* before mating and at piglet weaning.

The phylogenetic characterization into A, B1, B2 and D groups has been reported for several *E. coli* communities from animal hosts [4,10,11]. Johnson and Stell [12] observed that the extra-intestinal pathogenic strains from human were associated to phylogenetic group B2 and to a lesser extent to D, while commensals belonged to A. This phylogenetic characterization was also applied for evaluate extra-intestinal *E.coli* from animal source, and it was showed that A and B1 were associated with porcine pyelonephritis [4]. These two phylogroups together with D, were described in the bovine uterine colonization in normal puerperium as well in clinical metritis [13]. However, to our knowledge, there are no reports about the phylogenetic characterization of native *E. coli* populations from the urogenital tract of healthy cows and sows. More accurate descriptions of these microbial communities and their intrinsic virulence potential will allow a better understanding of their possible role in extraintestinal infections, as Johnson and Russo [14] suggested. Virulence profiles may be linked to phylogenetic groups as well as to antimicrobial resistance traits [15,16]. The resistance developed by *E. coli,* particularly extended-spectrum β-lactamase producers, is one of the main reasons for low cure rates for infections in livestock [17]. Moreover, both commensal and pathogenic *E. coli* in farming systems were recognised as reservoirs that can acquire and play a pivotal role in the transfer of antibiotic

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resistance [2,18,19]. Antimicrobials are administrated therapeutically, metaphylactically and prophylactically and also as growth promoters, strategies which are still applied in Argentina. Considering that *E. coli* belongs to the native microbiota of the vagina and urogenital tract of cows and sows respectively, and that some strains may be potential pathogens for the dam and its offspring, the aim of this study was to characterize native *E. coli* from these tracts in order to establish the phylogenetic structure, antibiotic resistance prevalence and the intrinsic virulence potential of these populations in healthy females.

2. Materials and methods

2.1. Animals and sampling

Eighty-two samples were taken from healthy females: 40 samples (vagina) from dairy (n = 26) and beef (n = 14) cows and 42 samples (urethra and vagina) from 21 sows housed in both, outdoor (n = 15) and indoor (n = 6) pig production systems. All these farms were located in Tucumán province, in the North-west of Argentina. Preparation and vaginal sampling collection were performed as previously described [20]. Urethral samples were obtained from sows by using a stainless steel speculum to access the meatus; a sterile cytobrush was then inserted and rotated on the urethral wall. Then, each cytobrush was placed in 1 ml phosphate buffered saline solution (PBS) containing tubes, pH 7.0, and kept refrigerated at 4 °C until processing.

2.2. Isolation and identification of autochthonous Escherichia coli

Microorganisms were dislodged from the cytobrushes (placed in PBS tubes) by vigorous agitation and cultured on MacConkey agar (Britania Laboratories, Argentina) at 37 °C (24 h). Lactose positive colonies (3–4 per plate of each sample) were sub-cultured for bacterial identification. They were subjected to standard biochemical tests (indole, methyl-red-Voges-Proskauer, citrate) for *E. coli* and confirmed by Polymerase Chain Reaction (PCR) for detection of the β-D-glucuronidase gene [11,21]. Primers sequences and annealing temperatures are indicated in Table 1, templates were prepared from a single colony (overnight culture on BHI agar, 37 °C) suspended in 50 µl sterile nuclease-free water (NFW) and boiled (10 min). The lysed products were centrifuged (800 × *g*, 10 min at 4 °C) and their supernatants were stored (–20 °C). The isolates were stored at −20 °C (BHI with 20% glycerol).

2.3. Phylogenetic group determination and detection of virulence genes

The isolates identified as *E. coli* were classified into four phylogenetic groups following the criteria proposed by Clermont et al. [22] based on three genetic markers: *chuA*, *yjaA* and TspE4.C2 (Table 1).

The occurrence of virulence genes associated with Attaching and Effacing (AEEC) and enterotoxigenic (ETEC) *E. coli* was evaluated by PCR assays. A standard PCR was applied to detect the *eae* (Intimin) gene [23]. Two fimbrial genes, F41 and F5 (K99), and the enterotoxin heat stable (STa) gene (associated with ETEC) were determined by a modified Franck et al. [24] multiplex protocol; *E. coli* EDL933 and B41 were used as quality controls.

2.4. Antimicrobial susceptibility of Escherichia coli isolates

The antimicrobial susceptibility was tested by the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI) [25]. The following antibacterial agents were tested: ampicillin (AMP, 10 µg), amoxycillin/clavulanic acid (AMC, 30 µg), ceftazidime (CAZ, 30 µg), ceftiofur (CEF, 30 µg), streptomycin (STR, 25 µg), enrofloxacin (ENR, 5 µg), tetracycline (TET, 30 µg), trimethoprim/sulphamethoxazole (SXT, 1.25/23.75 µg). All antimicrobials were purchased from Oxoid (UK). The references strains *E. coli* ATCC25922 and ATCC35218 were used as quality controls. The isolates were classified as sensitive (S), intermediate (I) and resistant (R), based on CLSI criteria. For the statistical analyses, isolates classified as intermediate (I) were considered resistant [26] and those with simultaneous resistance to three different antibiotic-classes were classified as Multiple Drug Resistant (MDR) [27].

2.5. Statistical analysis

Logistic regression was used to calculate the odds ratio (95% confidence interval). The differences in the resistance percentages from cattle and sows were analyzed (Fisher's exact test) in a 2 × 2 contingency table.

A contingency table was used to evaluate the relationship between phylogenetic group and antibiotic resistance profile or the occurrence of virulence genes (contribution to Chi-square) and a multivariate analysis of correspondence was performed to show the association between phylogenetic group and pathogenic characteristics. Data processing was carried out with MINITAB (version 14) and Infostat (2015p Version) software.

Table 1
Primers and PCR conditions for *E. coli* identification and phylogenetic/virulence characterization.

Gene	Primer secuence (5′-3′)	T° annealing	Size of products	Reference
uidA	TGTTACGTCCTGTAGAAAGCCC AAAACTGCCTGGCACAGCAATT	58 °C	154 bp	[21]
chuA	GACGAACCA ACGGTCAGGAT TGCCGCCAGTACC AAAGACA	58 °C	279 bp	[22]
yjaA	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	58 °C	211 bp	[22]
TspE4C	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	58 °C	152 bp	[22]
eae	GGAACGGCAGAGGTTAATCTGCAG GGCGCTCATCATAGTCTTTC	55 °C	775 bp	[23]
Gene encoding F41	GCATCAGCGGCAGTATCT GTCCCTAGCTCAGTATTATCACCT	50 °C	380 bp	[24]
Gene encoding F5 (K99)	TATTATCTTAGGTGGTATGG GGTATCCTTTAGCAGCAGTATTTC	50 °C	314 bp	[24]
Gene encoding STa	GCTAATGTTGGCAATTTTTATTTCTGTA AGGATTACAACAAAGTTCACAGCAGTAA	50 °C	190 bp	[24]

Table 2
Comparative prevalence of phylogenetic groups in cattle and sows.

	Number (%) of positive isolates		*P*-value (interspecies comparisons)
Phylo-group	Cattle	Swine	
A	51 (79.69*)	21 (80.77*)	1
B1	8 (12.5)	3 (11,54)	1
B2	0	0	
D	5 (7.81)	2 (7.69)	1
P-value (intergroup comparisons)	< 0.0002	< 0.05	

*Statistically significant ($p < 0.05$, Fisher's exact test).

3. Results

3.1. Escherichia coli identification and phylogenetic characterization

Eighty-two samples from cows (n = 40) and sows (n = 42) were analysed to investigate the presence of *E. coli.* Isolation of *Enterobacteriaceae* was positive in 31 (bovine) and 25 (porcine) samples. Also, 83.7% (n = 64) and 45.6% (n = 26) of the *Enterobacteriaceae* isolates from bovine and porcine groups, respectively, were confirmed as *E. coli.* Vaginal colonization of *E. coli* was statistically higher in cows (57.5%) than in sows (23.8%) (Fisher's exact test, $p \leq 0.05$). In addition, no significant differences were observed between vagina and urethra in sows (data not shown).

A total of 90 *E. coli* isolates (64 from cows and 26 from sows) were analysed by PCR and the results indicated that they belong to phylogenetic groups A, B1 and D, none of them was classified as B2. The prevalence of A was significantly higher ($p < 0.05$ Fisher's exact test) than B1 and D; however, there were no significant differences between B1 and D. Moreover, a similar distribution of the three phylogenetic groups detected was observed when interspecies comparisons were performed ($p > 1$, Fisher's exact test) (Table 2). Therefore, in both cows and sows, the majority of the isolates belonged to phylogenetic group A while the percentages of isolates belonging to B1 and D were significantly lower ($p < 0.05$ Fisher's exact test) (Table 2).

Although a similar phylogenetic structure was detected for cows and sows, some particular aspects related to farm management were observed. Thus, in dairy farms, a higher prevalence of B1 and lower of D were observed when compared to beef farms. In pig farms, a different distribution among the phylogenetic groups of indoor and outdoor systems was detected. Thus, 75% and 25% isolates from indoor farms belonged to phylogenetic group A and D, respectively; while in outdoor farms 83% of the isolates belonged to A and 16.67% to B1. Moreover, no isolate was identified as D.

3.2. Virulence profile

Ninety *E. coli* isolates were assessed to identify four intestinal virulence determinants and 14 (15.6%) showed at least one factor. The intimin gene (*eae*) was detected only among isolates from cows (n = 2, 3.13%) but no isolate from dairy cattle showed any of the virulence factors studied. The fimbrial F41 gene was detected in 5 (7.81%) of the isolates from cows and 2 (7.61%) from sows. Significant differences (Fisher's exact test $p < 0.05$) for the presence of the heat stable enterotoxin (STa) gene were observed between *E. coli* isolates from sows (19.23%, n = 5) and cows (3.13%, n = 2); however, no isolate was positive for the F5 fimbria gene. Only 2 isolates (2.22%), one from a cow and one from a sow, harboured genes coding for at least one adhesion factor and one toxin, which were F41 and STa, respectively.

3.3. Antimicrobial susceptibility and resistance

The prevalence of resistance to antimicrobial agents in both bovine and porcine isolates from each type of farm (dairy/beef in bovine and in/outdoor in porcine) is shown in Tables 3–5.

All *E. coli* isolates were susceptible to CAZ and all bovine isolates were susceptible to AMC and CEF. The results revealed statistically significant differences in the prevalence of resistance to AMP, AMC and STR. On the other hand, a low prevalence of SXT resistance was detected in *E. coli* from both groups (Table 3). Moreover, 61.54% of porcine isolates were resistant to at least one antibacterial agent, whereas 30.77% were MDR. Among bovine *E. coli*, 21.88% were resistant to at least one agent, while 4.69% were MDR (Table 3).

The results showed statistically significant differences in the distribution of isolates with resistance to at least one agent (p = 0.0005) as well as in the distribution of MDR isolates (p = 0.0017), they were higher among the swine isolates than among the bovine group (Table 3). When evaluating bovine isolates, the highest prevalence of resistance to at least one antimicrobial agent was observed in dairy cows isolates (p = 0.015) (Table 4). The prevalence of resistant and MDR isolates from sows did not show significant differences between indoor and outdoor farms (Table 5).

3.4. Phylogenetic groups versus virulence profile or antibiotic resistance

The correspondence analysis (Fig. 1) revealed an association

Table 3
Prevalence of resistance to antimicrobial agents among *E.coli* isolates from cows and sows.

	Number (%) of *E. coli* isolates			
Antimicrobial Agent	[a]Cattle	Swine	OR (95% CI)	*P*-value
Ampicillin (AMP)	5 (7.94)	11 (42.31)	0.12 (0.04–0.37)	0.0003*
Amoxicillin/Clavulanate (AMC)	0	5 (19.23)	–	0.0014*
Ceftazidime (CAZ)	0	0	–	
Ceftiofur (CEF)	0	1 (3.85)	–	0.2888
Streptomycin (STR)	7 (11.11)	9 (34.62)	0.23 (0.08–0.69)	0.0134*
Tetracycline (TET)	10 (14.29)	8 (30.77)	0.42 (0.15–1.19)	0.1451
Enrofloxacin (ENR)	4 (6.35)	3 (11.54)	0.51 (0.12–2.24)	0.4074
Trimethoprim/Sulphamethoxazole (SXT)	2 (3.17)	1 (3.85)	0.81 (0.10–6.43)	1
Antimicrobial Susceptibility Profile				
Resistant	14 (21.88)	16 (61.54)	0.18 (0.07–0.46)	0.0005*
Susceptible	50 (78.13)	10 (38.46)		
MDR	3 (4.69)	8 (30.77)	0.11 (0.03–0.43)	0.0017*
NMDR	61 (95.31)	18 (69.23)		

MDR: multidrug-resistant; NMDR: non-multidrug-resistant.
* Statistically significant ($p < 0.05$, Fisher-exact test).
[a] Reference category.

Table 4
Prevalence of resistance to antimicrobial agents among *E.coli* isolates from two bovine farming systems.

	Number (%) of *E. coli* isolates		
Antimicrobial Agent	[a]Dairy Farm (n = 18)	Beef Farm (n = 46)	OR (95% CI)
Ampicillin (AMP)	2 (11.11)	3 (6.52)	0.56 (0.10–3.11)
Streptomycin (STR)	3 (16.67)	4 (8.70)	0.48 (0.10–2.16)
Tetracycline (TET)	6 (33.33)	4 (8.70)	0,19 (0.05–0.74)*
Enrofloxacin (ENR)	1 (5.56)	3 (6.52)	1.19 (0.16–8.69)
Trimethoprim/Sulphamethoxazole (SXT)	0 (0)	2 (4.35)	–
Antimicrobial Susceptibility Profile			
Resistant	8 (44.44)	6 (13.04)	0.19 (0.06–0.64)*
Susceptible	10 (55.56)	40 (86.96)	
MDR	1 (5.56)	2 (4.35)	0.77 (0.09–6.30)
NMDR	17 (94.44)	44 (95.65)	

MDR: multidrug-resistant; NMDR: non-multidrug-resistant.
* Statistically significant ($p < 0.05$ Fisher-exact test).
[a] Reference category.

between the presence/absence of the virulence factor and the phylogenetic group, regardless of the origin of the isolates. Thus, *E. coli* with at least one virulence factor were classified as belonging to the D group. In contrast, there was a low probability that B1/A isolates had virulence factors associated with EPEC or ETEC (Fig. 1)

A similar correspondence analysis was applied to evaluate the association between antibiotic resistance profile and phylogenetic group (Fig. 2). Isolates with susceptibility to the evaluated antibiotics were only associated with the A group, while *E. coli* classified as D were related to resistance to at least one antibiotic but not to MDR. Moreover, MDR isolates were not related to any particular phylogenetic group.

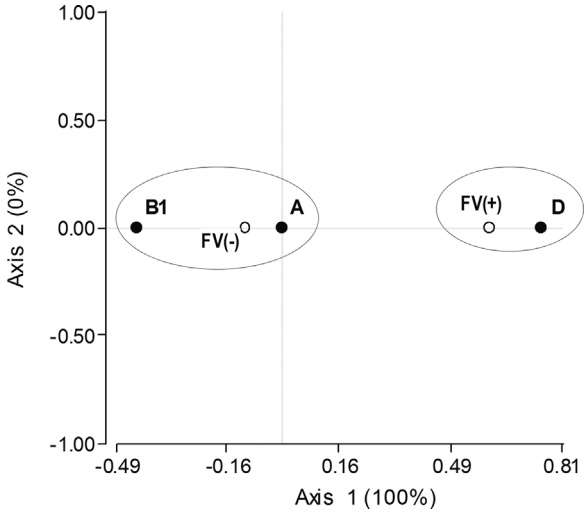

Fig. 1. Analysis of correspondence: biplot of phylogenetic groups and virulence factors harboured by *E. coli* isolates. The contribution to Chi-square is indicated in brackets. The occurrence or absence of virulence genes associated with attaching and effacing (AEEC) or enterotoxigenic (ETEC) *E. coli* were assessed as FV (+) or FV (−).

4. Discussion

The reproductive performance of female in farming systems is a key point that affects their productivity, and *E. coli* is recognized as an important pathogen that causes metritis in cows and urinary/uterine infections in sows [3,5]. Several studies have defined the microbial pathogenic profiles that represent a risk for these infections [7,28]; however, no research about the characteristics of autochthonous *E. coli* in the cited niches was found. In previous works, our group described the presence of *E. coli* in the native vaginal microbiota from healthy heifers and cows [8,20]. In the present work we are reporting a description of the phylogenetic structure of native *E. coli* populations from the vagina of healthy cows and urogenital tract of sows; also some virulent factors and antibiotic resistance prevalence were studied in order to evaluate if there is any difference regarding the farming systems examined. To the best of our knowledge, this is the first study describing these aspects in *E. coli* from native populations in these specific niches, which are infection targets with a key impact on the

Table 5
Prevalence of resistance to antimicrobial agents among *E.coli* isolates from sows of indoor and outdoor farming systems.

	Number (%) of *E. coli* isolates			
Antimicrobial Agent	[a]Indoor (n = 8)	Outdoor (n = 18)	OR (95% CI)	p-value
Ampicillin (AMP)	5 (62.50)	6 (33.33)	0.30 (0.06–1.55)	0.218307
Amoxicillin/Clavulanate (AMC)	3 (37.5)	2 (11.11)	0.21 (0.03 − 1.38)	0.280511
Ceftiofur (CEF)	0	1 (5.56)		1
Streptomycin (STR)	2 (25)	7 (38.89)	1.91 (0.34–10.71)	0.667285
Tetracycline (TET)	3 (37.5)	5 (27.78)	0.64 (0.12–3.40)	0.667285
Enrofloxacin (ENR)	3 (37.5)	0		0.0215385*
Trimethoprim/Sulphamethoxazole (SXT)	1 (12.50)	0		0.307692
Antimicrobial Susceptibility characteristic				
Resistant	7 (87.50)	9 (50)	0.14 (0.02–1.02)	0.0988526
Susceptible	1 (12.5)	9 (50)		
MDR	4 (50)	4 (22.22)	0.29 (0.05–1.53)	0.197202
NMDR	4 (50)	14 (77.78)		

MDR: multidrug-resistant; NMDR: non-multidrug-resistant.
* Statistically significant ($p < 0.05$, Fisher-exact test).
[a] Reference category.

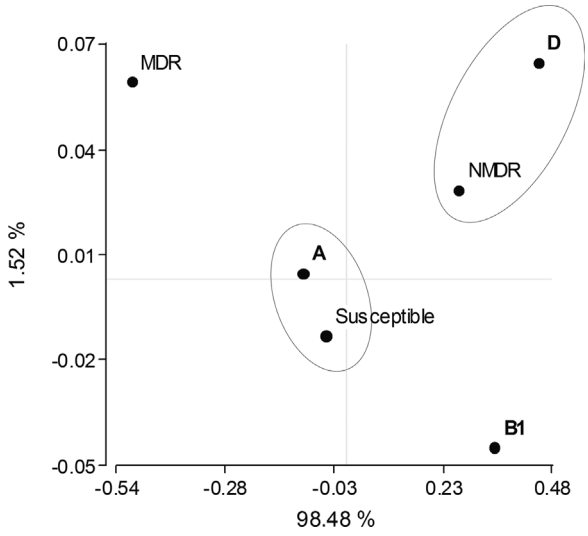

Fig. 2. Analysis of correspondence: biplot of phylogenetic group and antibiotic resistance profile of *E. coli* isolates. The contribution to Chi-square is indicated for each component. The susceptible isolates were sensible for all antibiotics assayed; those isolates showing antimicrobial resistance were classified as NMDR (non-multiple drug resistant) and those that exhibited simultaneous resistance to antimicrobial agents of at least three different classes were classified as MDR (multiple drug resistant).

reproductive performance of females in farming systems. Moreover, it is interesting to evaluate the presence of *E. coli* in the microbiota of these tracts, because they represent the main sources of microorganisms that will colonize the mucosa of the newborn [29]; as well as those that will access the uterus and cause metritis in the postpartum [30].

Even though *E. coli* was described as a constituent of the normal microbiota of the reproductive tract of healthy heifers [8,20] and sows [9,31]: none of these studies included a phylogenetic description. In the other hand, the presence and characterization of *E. coli* from metritic cows [7] and sows displaying pyelonephritis and dysgalactia has been widely reported [4,6]. However, to our knowledge there is no available information regarding the characterization of autochthonous *E. coli* from the urogenital tract of sows and vagina of cows. Therefore, in this work a meticulous sampling method of rigorously selected healthy animals was carried out to guarantee the isolation of commensal *E. coli* from the above mentioned mucosa. Among the *Enterobacteriaceae* population, our results demonstrated a higher proportion of *E. coli* isolated from cows than from sows. Similar results were reported by Mshelia et al. [32], who identified a high percentage of *E. coli* (72.5%) from bovine vaginal *Enterobacteriaceae* while Bara et al. [9] reported 22.5% of *E. coli* from vaginal samples of sows. Moreover, our results showed, for the first time, that there were no significant differences between *E. coli* colonization of vagina and urethra in sows.

We observed a similar phylogenetic conformation in the *E. coli* populations from healthy cows and sows which were mainly classified as A; but also B1 and D were detected; no isolates were assigned to group B2. A different phylogenetic composition was observed among the *E. coli* populations from uterus in postpartum, where the predominant group was B1 over A and D, with a high presence of *hlyE* (hemolysin E) and *hlyA* (α-hemolysin) genes [13]; probably because the B1 exhibits better capabilities to colonize in this postpartum environment and therefore, vaginal B1 *E. coli* should be studied in much more detail.

The presence of the majority groups A and B1 in the healthy porcine urethra was not a surprise, since it was observed that the pyelonephritis producer *E. coli* with both acute and chronic kidney lesions, belonged to group A, as well as to group B1 [4]; in our work hypothesis, the normal urogenital microbial communities include some *E. coli* populations which could be able to produce pyelonephritis.

In our study, the farming conditions of both beef and dairy cows did not affect the distribution of the phylogenetic groups present in vagina. However, Bok et al. [15] detected different phylogenetic structures in faecal *E. coli* populations from beef and dairy cattle; these observations may reflect a possible restriction or selection for the microbial colonization in the reproductive tract; which could be determined by specific conditions of these niches regardless of the environment. When assessing the phylogenetic characteristics among native *E. coli* from the urogenital tract of sows, some differences between indoor and outdoor farming conditions were observed; nevertheless, further studies should be carried out to determine if there are significant differences.

Enteropathogenic *E. coli* (EPEC) was reported as responsible for diarrhoea in farm animals such as pigs, and possesses an adhesin encoded by the *eae* gene named intimin [33]. On the other hand, the toxigenic pathotype (STEC) is characterized by the presence of intimin and Shiga-like toxin, implicated in oedema disease (ED) in piglets whereas in calves and lambs it is associated with diarrhoea and dysentery [33]. Based on these reports, we evaluated some intestinal virulence markers and observed a low prevalence of enteropathogenic factors among the *E. coli* isolates in this study. In trials performed in other species, like giant panda, the vaginal *E. coli* have not showed any virulence markers related to diarrheagenic pathotypes [11]. Our study represents the first report about the virulence factors, common to EPEC (intimin) and ECET (F5, F41 and STa) in autochthonous *E. coli* from bovine vagina and porcine vagina/urethra from healthy females. These characteristics should be taken into account since the first colonizers of the newborn animals will probably be these constituents of the native microbiota of the maternal reproductive tract [34].

Antimicrobial agents, often used in livestock, belong to the same classes than those administered in human treatments. Thus, the application of antibiotics in farms animals produces a selective pressure allowing the dissemination of resistant bacteria, which represents a risk to human health [19,35]. Several investigations highlighted the occurrence of antibiotic resistance in native intestinal *E. coli* isolates from both bovine and swine farming systems [15,18,36,38,39]. However, we have not found studies addressing antibiotic resistance in native urogenital *E. coli* from healthy mothers on these types of farms.

When comparing the antibiotic resistance in both, pigs and cattle, our finding showed greater percentage of sensible *E. coli* among bovine (78.13%) than in the porcine (38,46%) group. This tendency was also observed in a polish study for fecal *E. coli* isolated from sows/piglets and dairy cows [38]. The different levels of resistance probably are due to differences in the niche conditions and the technology used in the farming systems. Furthermore, our results showed higher MDR prevalence among porcine *E. coli* than in bovine. This observation could be a consequence of the management protocols applied in pigs farming, which are used worldwide and allow to permanent exposure of the animal microbiota to sub-therapeutic doses of different antibiotics [39]. Studies performed in other regions of Argentina, with greater level of technology in pig farming than ours, showed high MDR in fecal *E. coli*, being 72% in newborn piglets and 94,7% in fattening animals [40]. Our results show that MDR *E. coli* are not exclusive to the intestinal microbiota and that even in modest farming, the resistance prevalence of potential veterinary pathogens of the reproductive tract, should not be undervalued.

When evaluating the bovine isolates, the percentage of resistant *E.coli* derived from dairy cows was higher than from beef cows; similar finding were observed by Bok et al. [15]. They concluded that fecal *E. coli* isolates with a high level of antimicrobial resistance are more prevalent in intensive farms. This does not come as a surprise because it is well known that antibiotic therapies are more frequents in dairy than in beef farming systems, in this region of Argentina.

Also, as we expected, the percentage of tetracycline resistance was significantly high for the dairy bovine isolates, since it has been previously reported that it is commonly applied in cattle not only for the control and treatment of diseases, but also as a growth promoter [37]. Furthermore, in Argentina, even though the use of antibiotics as feed additives will be banned from 2019 onwards [41], several antibiotic-

containing products are still available in local veterinarian supplies markets.

The results presented here show that the occurrence of resistance among the native *E. coli* isolated from the healthy vagina in dairy cows, was to tetracycline, streptomycin, ampicillin and enrofloxacin. If we think that the vaginal microbiota could harbor potential endometrial-pathogenic *E. coli*, it is interesting to highlight that isolates from uterus of cows with metritis/endometritis, have shown prevalence of above 30% for ampicillin, streptomycin and oxytetracycline [42].

The impact of antibiotic use in the swine industry has been extensively discussed [37]; however it has not been evaluated in this microbial niche; and our findings indicated the presence of resistance to the same antibiotics previously described in pork [18]; therefore, the microbiota of the reproductive tract should be under the same level of surveillance than the intestinal one.

A correspondence analysis allowed us to conclude that the phylogroup A was related to the susceptible and D to NMDR. However, no phylogenetic group was exclusively associated with MDR. Lay et al. [16] reported that more than 98% of intestinal *E.coli* from healthy swine were MDR and belonged to group B1 but none to phylogroup A. Another study described that 50% of porcine pyelonephritic *E. coli* were resistant to four or more antibiotics and the 90% belonged to B1 [43]. Our analysis demonstrated significant differences: B1 isolates showed resistance against several antibiotics and none from the A group was resistant to sulphamethoxazole/trimethoprim; only one from this latter group was resistant to ampicillin. Overall, the results presented as well as the previous evidences, suggest that *E. coli* from phylogroup A might have a limitation to acquire antibiotic resistance.

5. Conclusions

This work represents the first characterization of autochthonous *E. coli* from vagina of healthy cows and from urogenital tract of healthy sows regarding the association between phylogenetic profile, virulence factors and antimicrobial resistance; moreover, these results contribute to the knowledge of extra-intestinal *E. coli* populations, which could be implicated in the reproductive health of the females in farms.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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