



Research article

Antimicrobial resistance in *E. coli* isolated from dairy calves and bedding material

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ABSTRACT

Introduction: *E. coli* is a ubiquitous bacterium commonly used as a sentinel in antimicrobial resistance studies. Here, *E. coli* was isolated from three groups (sick calves, healthy calves and bedding material), to assess the presence of antimicrobial resistance, describe resistance profiles, and compare these resistances among groups.

Material and methods: Samples were collected from calves and calving pens from 20 dairy farms. Using the disc diffusion method, *E. coli* isolates were screened for antimicrobial resistance against seven antimicrobials: Amoxicillin, Ceftiofur, Gentamicin, Enrofloxacin, Trimethoprim-sulfamethoxazole, Florfenicol and Oxytetracycline. Isolates resistant to all these seven antimicrobials were tested again against an extended 19 antimicrobial drug panel and for the presence of the most common *E. coli* pathogenicity genes through PCR.

Results & discussion: Three hundred forty-nine *E. coli* isolates were obtained; most isolates were resistant to a single antimicrobial, but 2.3% (8) were resistant to 16 to 19 of the antimicrobials tested. The group with the highest percentage of multiresistant isolates was the calves with diarrhea group. Younger calves provided samples with higher antimicrobial resistance levels.

Conclusions: There is a high rate of antimicrobial resistance in dairy farms calving pens. These bacteria could not only be a resistance gene reservoir, but also could have the potential to spread these determinants through horizontal gene transfer to other susceptible bacteria. Measures should be taken to protect colonization of younger calves, based on hygienic measures and proper management.

Interpretive summary

The evaluation of bacteria isolated from calves in dairy farms allowed for the identification of isolates resistant to multiple antimicrobials from different families. These, although coming from non-aggressive bacteria, pose a risk for public health being potential reservoirs for antimicrobial resistance genes for the microbial community.

1. Introduction

Nowadays, antimicrobial resistance is one of the most significant threats to human and animal health [1, 2, 3], and animal production systems have been pointed out as one of the most relevant hotspots of antimicrobial resistance generation and dissemination [4, 5, 6]. Evidence suggests that the incorrect use of antimicrobials in animal production is the most important factor for the emergence, selection, and dissemination of antimicrobial resistance [7, 8]. Some of the practices associated to the misuse of antimicrobials are wrong diagnostics, erroneous treatment schemes, incorrect dosage, or non-compliance of required treatment periods [9].

In dairy systems, antimicrobials are commonly used in cows for the

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treatment of some diseases such as mastitis, metritis and lameness, as well as a prophylactic treatment for mastitis before the dry period starts [5]. As a consequence, milk produced during treatment could be contaminated with antimicrobial residues [10, 11]. In some cases, and as a way to reduce economic losses due to the inability to sell it, this milk is used to feed calves [12, 13, 14]. Research has demonstrated that the selection of resistant bacteria can take place at sub-minimum inhibitory concentrations, facilitating the occurrence of antimicrobial resistance [15, 16]. *E. coli* is a bacterial species frequently used as a sentinel for antimicrobial resistance [17]. This microorganism is widely distributed in the intestinal microbiota of animals and can easily acquire mobile genetic elements such as those that encode antimicrobial resistance due to its genomic plasticity [18].

In Chile, antimicrobial resistance in livestock has been addressed sparsely in the last decades. Previous works have analyzed cattle feces in 2002, cow milk in 2005, and recently some researchers have studied antimicrobial resistant bacteria from necropsy of dairy cattle in southern Chile [10, 19, 20]. These studies have indicated the extensive presence of antimicrobial resistance and multiresistant bacteria in dairy cattle. Recently, Chilean authorities have established a comprehensive program to address the issue of antimicrobial resistance at the national level including animal husbandry among their targets [21].

The main goal of this study is to measure the antimicrobial resistance in *E. coli* obtained from healthy and sick calves, and from calving pens—as a proxy of the close environment of the sampled animals—to better understand the antimicrobial resistance in early stages of dairy production.

2. Materials and methods

2.1. Farm selection and sample collection

Sample size was determined to be of 95 *E. coli* isolates per group considering a 95% confidence and estimating a 20% difference between the three groups (sick calves, healthy calves and environmental samples) [22]. To obtain the required isolates, a convenience sampling of 20 dairy farms from Los Ríos and Los Lagos regions in southern Chile was used. These are the two most important dairy production regions in Chile. Sampling only included farms with more than 100 dairy cows to aim for more industrial level farms which have better facilities and records. From each farm, a total of 20 samples were collected from calves and from the calving pen bedding material in each visit; samples included 10 calves with diarrhea, 5 healthy calves, and 5 randomly selected samples from the floor material of pens in use. Calves with diarrhea were selected using a targeted sampling strategy based on clinical signs and the opinion of the technician in charge of the animals. Healthy calves were selected randomly using a random number table, and the same procedure was used to select calving pens to sample. The sampling only considered calves that were not subjected to antimicrobial therapy. Samples from calves were collected through rectal fecal grab using sterile gloves. This procedure was conducted according to bioethical standards dictated by the OIE [23]. Bedding material samples were directly collected from the floor and stored in a sterile container. Both samples, from calves and bedding material, were stored in sterile 50 ml containers and cooled in ice prior to transportation to the laboratory within 8 hrs. This study was approved by the Faculty of Veterinary Sciences from University of Chile's Ethics Committee, Protocol number 17-2014. Individual animal information was collected at sampling, and it included breed, age, sex and clinical signs.

2.2. *E. coli* isolation and antimicrobial resistance

For *E. coli* isolation, 5 g of sample were homogenized with 10 ml of sterile 0.85% NaCl, and 10 µL were directly cultured in MacConkey Agar (BD, NJ) with a sterile inoculating loop. Plates were incubated at 35 ± 2 °C for 24–48 h. After incubation, one or two lactose (+) colonies were

collected, and biochemical identification was performed to confirm *E. coli* colonies using IMViC test, and results were interpreted using the Bergey's manual [24]. For atypical colonies, DNA was extracted using the boiling method [25] and tested with conventional PCR using previously reported primers [26]. Confirmed isolates were stored at -80 °C in Brain Heart broth + 20% glycerol until used.

Antimicrobial resistance screening test was performed in all presumptive *E. coli* isolates; the Kirby-Bauer disc diffusion method was performed in Mueller-Hinton Agar according to the Clinical and Laboratory Standards Institute [27]. In brief, a suspension of a fresh culture was prepared in saline solution at 0.85% and adjusted to a McFarland 0.5 turbidity. Then, the suspension was inoculated into a Mueller-Hinton Agar plate, and discs with different antimicrobials were positioned over the inoculated plate (Table 1). After incubation at 35 ± 2 °C for 16–18 h inhibition halos around each disc were measured. *E. coli* ATCC® 25922 was used as quality control during the procedure [27]. The seven different discs (OXOID®; Table 1) were chosen based on the most commonly used antimicrobials in cattle in Chile and on local experts recommendations [28]. Breakpoints were determined using the CLSI guidelines [27].

Isolates displaying resistance to all seven antimicrobials were further tested for additional 12 antimicrobials using the disc diffusion method (Table 2). This resulted in 19 antimicrobials evaluated for this isolate sub-group. To define the potential pathogenicity of the resistant isolates, a PCR described by Franck *et al.* was performed to the same sub-group, detecting genes from Enterotoxigenic *E. coli* (ETEC) and Shigatoxin producing *E. coli* (STEC) which cause diarrhea in calves. The PCR detected genes for F41 fimbriae (*fimF41*; ETEC), fimbriae subunit (K99, ETEC), heat stable enterotoxin a (*sta*, ETEC), shigatoxin 1 (*stx1*; STEC), and shigatoxin 2 (*stx2*; STEC) [29].

2.3. Data analyses

Microbiology results were recorded in an Excel® spreadsheet and combined with survey data. This allowed for crossed analyses and interpretation of all information available. For statistical and epidemiological evaluations, Infostat® Software Professional edition was used. Univariate analyses (Kruskal-Wallis and Pearson correlations) and descriptive statistics were performed for all the data. Results were aggregated in tables and graphics for easier interpretation. To generate comparable information on the number of antimicrobial resistant isolates per age group, a simple score was generated: A resistant result received a 1, whereas a susceptible test received a 0. All isolates were tested 7 times (one time per antimicrobial), and an average was calculated. As a result, each isolated received a final score of between 0 (an isolate fully susceptible to all 7 antimicrobial tested) to 1 (isolate resistant to all 7 antimicrobials tested). These scores were used to summarize results in association with the farms that originated the samples and the age of the calves (Figs. 2 and 3).

Table 1

Antimicrobials tested in the diffusion disc test for *E. coli* isolates obtained from calves. (n = 349).

Antimicrobial family	Antimicrobial (Abbreviation)	Content µg per disc
β-Lactams	Amoxicillin (AMX)	10
	Ceftiofur (CEF)	30
Aminoglycosides	Gentamicin (GEN)	10
Quinolones	Enrofloxacin (ENR)	5
Sulphonamide complex	Trimethoprim-sulfamethoxazole (SXT)	1,25/23,75
Phenicol	Florfenicol (FLO)	30
Tetracyclines	Oxytetracycline (OXT)	30

Table 2
Extended phenotypical antimicrobial resistance test to the seven most resistant *E. coli* isolates from dairy calves (n=7).

Antimicrobial family	Sensidiscs (Abbreviation)	Isolate ID						
		04-02	05-15	09-11	12-06	15-18	15-10	16-03
β-Lactams	Ampicillin (AMP)	R	R	R	R	R	R	R
	Amoxicillin (AMX)	R	R	R	R	R	R	R
	Amoxicillin-clavulanic acid (AMC)	S	S	S	S	S	S	S
	Ceftiofur (CEF)	R	R	R	R	R	R	R
	Cefoxitin (FOX)	S	S	S	S	S	S	S
	Ceftriaxone (CRO)	R	R	R	S	R	R	R
	Imipenem (IPM)	S	S	S	S	S	S	S
Aminoglycosides	Streptomycin (STR)	R	R	R	R	R	R	S
	Gentamicin (GEN)	R	R	R	R	R	R	R
Quinolones	Ciprofloxacin (CIP)	R	R	R	S	R	R	R
	Enrofloxacin (ENR)	R	R	R	R	R	R	R
Sulphonamide complex	Nalidixic acid (NA)	R	R	R	R	R	R	R
	Trimethoprim-sulfamethoxazole (SXT)	R	R	R	R	R	R	R
Phenicols	Sulfisoxazole (SFX)	R	R	R	R	R	R	R
	Chloramphenicol (CLO)	R	R	R	R	R	R	R
Tetracyclines	Florfenicol (FLO)	R	R	R	R	R	R	R
	Tetracycline (TET)	R	R	R	R	R	R	R
Macrolides	Oxytetracycline (OXT)	R	R	R	R	R	R	R
	Azithromycin (AZM)	R	S	S	S	S	S	R

Antimicrobial resistance tested with the diffusion disc test methodology following the CLSI methodology and cut off points [27].

19 antimicrobials were tested for 7 strains resistant to the panel in Table 1.

R: Resistant, S: susceptible. Ampicillin (AMP), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ceftiofur (CEF), cefoxitin (FOX), ceftriaxone (CRO), imipenem (IPM), streptomycin (STR), gentamicin (GEN), ciprofloxacin (CIP), enrofloxacin (ENR), nalidixic acid (NAC), trimethoprim-sulfamethoxazole (SXT), sulfisoxazole (SFX), chloramphenicol (CLO), florfenicol (FLO), tetracycline (TET), oxytetracycline (OXT), azithromycin (AZM).

3. Results

In this study, we analyzed the antimicrobial resistance of *E. coli* isolates obtained from calves' feces and calves' pen bedding material. Overall, 349 *E. coli* isolates were obtained from 400 samples: 194 isolates were obtained from calves with diarrhea, 96 from healthy calves, and 59 from bedding material. Different percentages of resistance were detected. Most of the isolates were resistant to amoxicillin (92%, 321/349), and over 53% were resistant to oxytetracycline (53.6%; 187/349). Lower resistant percentages were seen against ceftiofur (18.3% 64/349) and gentamycin (7.2% 25/349) (Table 3).

Large variations in the number of antimicrobial resistances displayed by individual isolates were also observed. Only 16 of 349 isolates (4.6%) were susceptible to all 7 tested antimicrobials (Fig. 1). Most of the isolates were resistant to one antimicrobial (33.6%); while the majority of these isolates were originally obtained from healthy calves and bedding material. Oppositely, most isolates resistant to two to six antimicrobials were obtained from calves with diarrhea. Isolates resistant to all seven antimicrobials (2.3%; 8/349) were isolated from calves with diarrhea and in the bedding material. Moreover, we detected differences among the 20 participating farms; there were farms where isolates were mostly resistant to more than 4 antimicrobials while in others, resistance to one or two antimicrobials were more frequent (Fig. 2).

It was also detected a wide variety of antimicrobial resistance patterns. Over 60% of isolates showed resistance to between one and three antimicrobials (Table 4). Isolates displaying resistance to four antimicrobials showed six different patterns. The same was observed for isolates

Table 3
Percentage of antimicrobial resistance in *E. coli* obtained from calves from dairy farms (n = 349).

Antimicrobial	Diarrhea calves	Healthy calves	Bedding material	Average resistance
Amoxicillin	95%	86%	90%	92%
Ceftiofur	21%	13%	20%	18.3%
Enrofloxacin	36%	13%	25%	27.5%
Florfenicol	32%	18%	15%	25.5%
Gentamicin	9%	4%	7%	7.2%
Oxytetracycline	64%	42%	37%	53.6%
Trimethoprim-sulfamethoxazole	48%	22%	29%	37.5%

displaying resistance to five antimicrobials (Table 4). All isolates that showed resistance to either four or five antimicrobials, included resistance to amoxicillin. Isolates resistant to six antimicrobials formed four different resistance patterns, and trimethoprim-sulfamethoxazole resistance was common to all.

To further investigate antimicrobial resistance in *E. coli* obtained from early dairy production systems, we tested seven of the isolates that were resistant to the seven antimicrobials in the first panel, against 12 additional antimicrobials, completing a 19 antimicrobial panel (Table 2). Results indicated that twelve antimicrobials were ineffective for all seven isolates including drugs of almost every antimicrobial family tested (β-Lactams, aminoglycoside, quinolones, sulphonamides, phenicols, tetracycline, and macrolides). Conversely, all seven isolates were susceptible to three β-Lactams: amoxicillin-clavulanic acid (AMX), cefoxitin (FOX), and imipenem (IPM) (Table 2). Finally, six of the isolates were resistant to all quinolones, except by isolate 12-06 which was susceptible to ciprofloxacin. Noteworthy is that multiresistant isolates were originated from six different farms. Additional analyses of these isolates revealed that they did not carry the tested virulence genes (*K99*, *fim*, *F41*, *sta*, *stx1*, *stx2*) suggesting they were non-pathogenic *E. coli* strains [29].

Statistical analyses were performed to search for associations between epidemiological factors and antimicrobial resistance. Statistical differences were not found when comparing percentages of antimicrobial resistance between isolates obtained from animals of different sexes or among breeds (data not shown). A low to moderate negative significant correlation was observed when associating age and antimicrobial resistance (Spearman correlation -0.3, $p < 0.001$), showing that isolates obtained from younger calves were more resistant than isolates from older calves, and a resistance peak at five to six weeks old calves (Fig. 3). We also found that a higher percentage of isolates were resistant to 3 to 7 antimicrobials in calves with diarrhea than in calves without diarrhea and bedding pen material where most isolates were resistant to 1 or 2 antimicrobials (Fig. 1).

4. Discussion

E. coli strains are considered an important potential reservoir of antimicrobial resistance genes in food producing animals, and it is frequently used as a sentinel for antimicrobial resistance [17]. In this

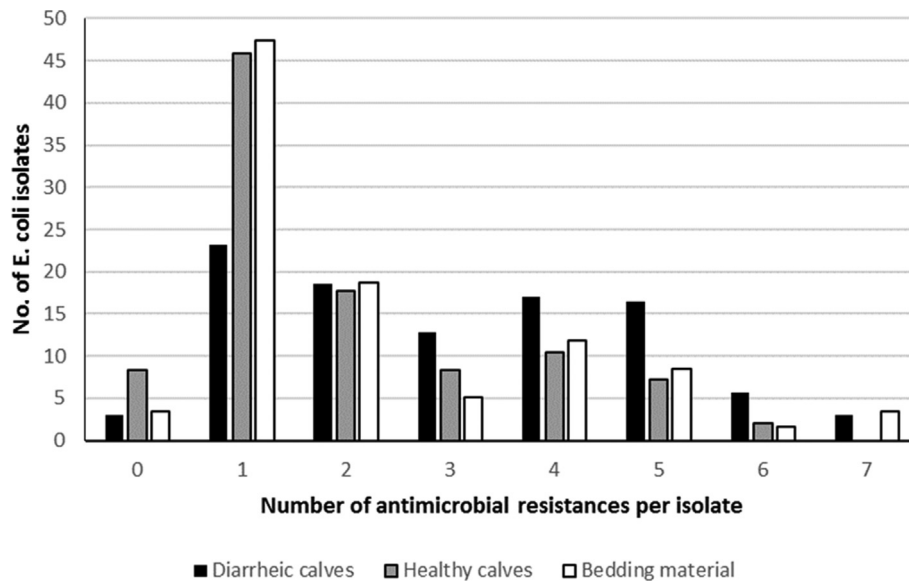


Fig. 1. Percentage of *E. coli* isolates resistant to 0 to 7 antimicrobials from diarrheic calves, healthy calves and isolates from bedding material. All percentages in each category (Diarrheic, Healthy and bedding) add to 100.

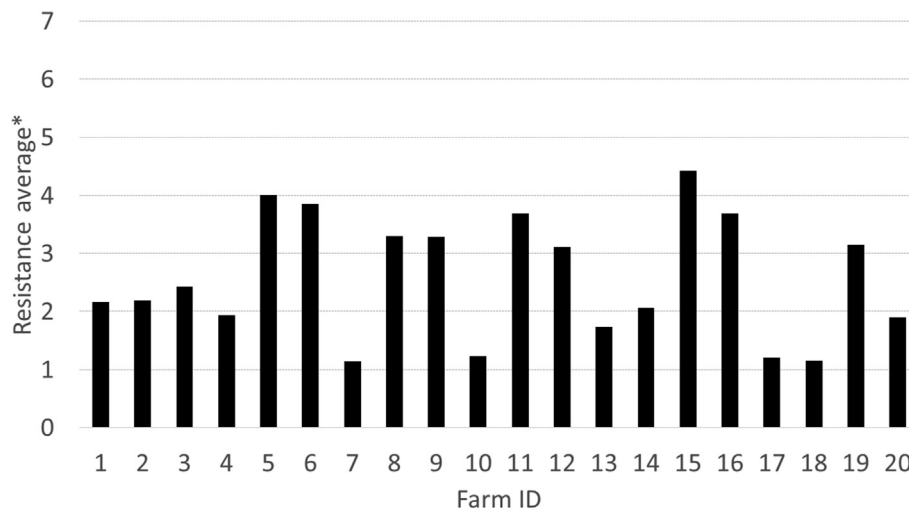


Fig. 2. Antimicrobial resistance percentage in calves and calving pens per farm. *Each farm had a variable number of *E. coli* isolates tested for seven antimicrobials. Therefore each isolate could receive a score of between zero (no resistances found) to seven (resistant to all seven antimicrobials tested). The score was averaged per farm.

study, we isolated resistant *E. coli* with distinct antimicrobial resistance patterns and in different percentages from calves and bedding material from 20 different farms.

Analysis of the pattern of antimicrobial resistance of 349 isolates, obtained from healthy and sick (diarrheic) calves as well as in the bedding material, allowed us to determine that antimicrobial resistance is a widespread phenomenon in Southern Chile where data about this topic is scarce. Interestingly, we identified that over one third of the isolates were resistant to more than three different families of antimicrobials, and that multiresistant isolates (isolates non-susceptible to all seven families of antimicrobial drugs tested) were mainly found in animals suffering diarrhea (Fig. 1). The explanation to this phenomenon is not clear, in special since none of the calves sampled were under antimicrobial treatment. However, it is possible that some animals were under treatment, but records were missing, and the treatment failed to be reported. Some authors have observed similar results in non-treated animals, and they explained this situation by the presence of pathogenic pathotypes with increased antimicrobial resistance genes that

could be jointly expressed, as reported in diarrhea in piglets [30, 31]. Even so, none of the seven isolates evaluated in the extended antimicrobial resistance panel and PCR showed the presence of any of the classical virulence genes of pathogenic *E. coli* pathotypes for calves. In addition, the different farms from which these isolates were obtained, were not linked in any noticeable way and therefore is hard to imagine the potential spread of one or many pathotypes between farms. Moreover, it has been shown that very young animals do not have a fully functional intestinal microbiota [31]. This, accompanied with a constant consumption of sub-MIC residues might result in intestinal colonization by multiresistant or pathogenic bacteria [15]. These results are in accordance with the findings shown in Fig. 3 where we detected a variation in antimicrobial resistance percentages in association to the age of the animal samples. There is a peak in antimicrobial resistance at the fifth - sixth weeks of age, and that afterwards resistance percentages were gradually reducing. This has been previously reported by Duse et al. and Pereira et al. [13, 15].

Additionally, we observed disparate percentages of resistance against

Table 4
Phenotypical antimicrobial resistance of *E. coli* isolated from dairy calves and bedding environment.

Antimicrobial resistance profile	Isolate No.	Isolate %
CEF; ENR; AMX; SXT; GEN; FLO; OXT	8	2,3
ENR; AMX; SXT; GEN; FLO; OXT	5	1,4
CEF; ENR; AMX; SXT; GEN; FLO	4	1,1
CEF; ENR; SXT; GEN; FLO; OXT	4	1,1
CEF; ENR; AMX; SXT; GEN; OXT	1	0,3
ENR; AMX; SXT; FLO; OXT	22	6,3
CEF; ENR; AMX; SXT; OXT	13	3,7
CEF; AMX; SXT; FLO; OXT	4	1,1
AMX; SXT; GEN; FLO; OXT	3	0,9
CEF; ENR; AMX; SXT; FLO	1	0,3
ENR; AMX; GEN; FLO; OXT	1	0,3
ENR; AMX; SXT; OXT	18	5,2
AMX; SXT; FLO; OXT	12	3,4
CEF; AMX; SXT; OXT	9	2,6
ENR; AMX; FLO; OXT	6	1,7
AMX; SXT; GEN; FLO	3	0,9
CEF; ENR; AMX; OXT	2	0,6
AMX; SXT; OXT	21	6
AMX; FLO; OXT	5	1,4
CEF; ENR; AMX	3	0,9
ENR; AMX; OXT	2	0,6
CEF; AMX; SXT	1	0,3
CEF; AMX; FLO	1	0,3
ENR; AMX; FLO	1	0,4
AMX; SXT; FLO	1	0,5
CEF; AMX; OXT	1	0,6
One or two resistances	181	51,2
Fully sensitive	16	4,6

Isolates were tested through the disc diffusion technique following the CLSI guidelines.

Abbreviations: Ceftiofur (CEF), Enrofloxacin (ENR), Amoxicillin (AMX), Trimethoprim-sulfamethoxazole (SXT), Gentamicin (GEN), Florfenicol (FLO), Oxytetracycline (OXT).

individual antimicrobials. For example, the high percentage of resistance to oxytetracycline described in this study agrees with previous reports from *E. coli* isolates from the same area [10, 20]. Moreover, resistance to tetracycline in *E. coli* isolates from fecal samples has also been reported in other countries such as Sweden and the United States of America [15, 31]. Nowadays, this drug is considered as critical for its use in veterinary medicine by the World Animal Health Organization [34] based on its broad spectrum and multiple application forms.

Quinolones are a group of antimicrobials frequently used in animal

production and for the treatment of human infections in Chile [35]. Interestingly, we found that 36% of *E. coli* isolates were resistant to enrofloxacin, one of the most used quinolones in Chile. This percentage is high, but lower than what has been found in previous unreported studies. Among the seven most resistant strains found, all of them were resistant also to the other two quinolones tested (nalidixic acid and ciprofloxacin). Antimicrobial resistance to quinolones is particularly significant because there is evidence of cross resistance with other antimicrobials relevant for human medicine [10, 36]. Furthermore, an increase in resistance to quinolones has been reported in Latin America [37], and antimicrobial resistance against quinolones has increased in *Salmonella* isolated in Chile [38] and in the United States [39]. Consequently, resistance to quinolones has been classified in the WHO priority list [40].

Antimicrobial resistance percentages to gentamicin and ceftiofur were low in tested *E. coli* isolates (7.2 and 18.3% resistance respectively). Large variations have been reported about resistance to these two antimicrobials. For instance, San Martin *et al.* [10] found 54% isolates were resistant to ceftiofur in *E. coli* isolates from dairy cattle feces. Those results are much higher than previous reports from mammary pathogens showing resistance percentages close to 7% [19]. In the United States, Pereira *et al.* [16], reported 100% susceptibility to gentamicin, whereas 48% of isolates were ceftiofur-resistant in *E. coli* isolates from fecal samples of cattle. In Sweden, a similar study found that 100% of isolates were susceptible to both antimicrobials [32]. It is important to note that resistance to β -lactams, and specially to third generation cephalosporin, like ceftiofur, is of great importance for human medicine and it is also considered as of critical importance for human health by the WHO [40]. Therefore, the 18% resistance to ceftiofur plus the 92% resistance to amoxicillin could imply a potentially serious threat to public health.

When observing the antimicrobial resistance patterns identified, it was found that one of the most common combinations was the one between Trimethoprim-sulfamethoxazole and oxytetracycline, generally associated to resistance to other antimicrobials (Tables 2 and 3). This combination was found in 123 of 152 multiresistant isolates (81%), thus strengthening the idea presented by some authors where bacteria with resistance to sulfonamides, tetracycline and streptomycin may have a selective advantage over other bacteria in the intestinal tract in calves younger than 3 months [32, 41, 42].

Multiresistant strains, defined as isolates that lost susceptibility to drugs in three or more antimicrobial families, is an increasing problem worldwide [43]. In this study, almost 20% of *E. coli* isolates were resistant to five or more antimicrobials. In a previous study in Sweden, 28% of

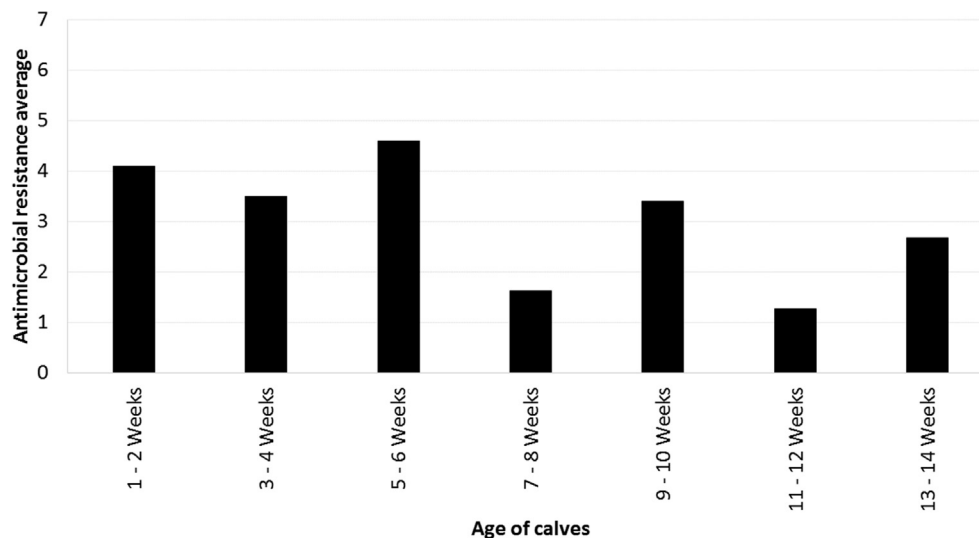


Fig. 3. Antimicrobial score average found per isolate from diarrheic and healthy calves distributed per age of calves. *Each farm had a variable number of *E. coli* isolates tested for seven antimicrobials. Therefore each isolate could receive a score of between zero (no resistances found) to seven (resistant to all seven antimicrobials tested). The score was averaged per calves' age group.

E. coli isolates obtained from dairy calves were resistant to three or more antimicrobials [32]. In a local study by San Martín *et al* [10], 56% of *E. coli* isolates from dairy cattle were resistant to over three antimicrobials, and 8% were resistant to five or more antimicrobials. These results are in line with all these global findings and confirms that Chile is not special in regard to multiresistant strains in dairy calves.

In our study, we confirmed bacterial identity of most isolates by biochemical tests and did not use other techniques as PCR. This might result in that some of the isolates obtained could not be actual *E. coli* but other coliforms; however, evidence demonstrate that a good correlation exists between both techniques [44]. Therefore, the impact of such drawback might not be that relevant in our results. Importantly, we confirmed that all seven multiresistant isolates (the ones that were resistant to all seven initially tested antimicrobials) were *E. coli* through both techniques. In addition, we used the diffusion disc test to assay for antimicrobial resistance in all *E. coli* isolates. The Gold standard to evaluate antimicrobial resistance is the minimum inhibitory concentration test [45], however the processing of a large amount of isolates implies the use of automatic instruments and large amount of antibiotics, which were not available. Conversely, the methodology selected for this study allows for an adequate approximation to the issue [27]. The diffusion disc test is endorsed by the CLSI and delivers valuable information in a cost-effective solution and providing the opportunity to analyze multiple isolates and reaching multiple farms.

It is also important to note the potential relevance of the environment in the maintenance and diffusion of antimicrobial resistance. In this study, the same resistance patterns were found in both groups of animals (sick and healthy) and their environments in different farms. This raises the hypothesis that the environment may be playing a key role as a reservoir for antimicrobial resistance for animals [46], and defining that normal farming conditions are compatible with the support and generation new antimicrobial resistant bacteria ready to colonize housed animals.

Finally, it has been widely demonstrated that the use of milk with antimicrobial residues to feed calves can favor the emergence and diffusion of antimicrobial resistance in the animal digestive system [11, 16] and previous research has demonstrated that this practice considerably increases antimicrobial resistant bacteria in calves' feces. Therefore, this practice increases the relative number of antimicrobial resistance elements which could easily transfer between bacteria [8, 16]. The use of discarded milk for calves feeding remains to be evaluated in the Chilean dairy farms setting and was not addressed by this study.

5. Conclusion

These results bring awareness of a serious issue in the studied farms, considering the high antimicrobial resistance percentages found. Also, it was found that the first weeks of age is a critical period for calves, were there are more susceptible to acquiring multiresistant isolates, most likely from the environment. Therefore, hygiene and proper management could be the key to reduce exposure to calves, moreover, considering the evidence of multiresistant isolates in the close environment of the animals.

Our data show high percentages of antimicrobial resistance in *E. coli* isolates obtained from dairy calves. These bacteria could be reservoirs of resistance genes and have the potential to spread these determinants to other susceptible bacteria. Thus, it is important to implement effective control measures (management, hygiene, treatment records, etc.) to reduce emergence and spread of antimicrobial resistant *E. coli* in dairy farms.

Declarations

Author contribution statement

Francisco Astorga, María P. Miro, Verónica Bravo, Magaly Toro:

Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

María J. Navarrete-Talloni: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Carlos J. Blondel: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Luis P. Hervé-Claude: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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