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Conference Proceedings Paper

Presence of antibiotic-resistant *Enterococcus faecalis* in colostrum supplied to calves? †

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†Presented at the 1st International Electronic Conference on Antibiotics – The Equal Power of Antibiotics and Antimicrobial Resistance, 08-17 May 2021; Available online: https://eca2021.sciforum.net

Abstract: Colostrum can be responsible for colonizing calves gastrointestinal tract by antibiotic-resistant bacteria, such as *Enterococcus faecalis* one of the main indicators of fecal contamination and associated with nosocomial infections. In this work, the aim to characterize antibiotic resistance in *E. faecalis* isolates from colostrum used in the feeding of calves. Nineteen one isolates were recovered using agar selective plates and confirmed by biochemical and genetic tests. The antimicrobial susceptibility was performed using 14 antimicrobial agents by the disk diffusion method, according to the Clinical and Laboratory Standards Institute standards. All *E. faecalis* isolates were intrinsically resistant to quinupristin-dalfopristin. The majority showed antibiotic-resistance to tetracycline (79.1%), erythromycin (79.1%) and streptomycin (57.1%). Isolates were less resistant to rifampicin (47.3%), chloramphenicol (25.3%) and ciprofloxacin (11.0%). Resistance to the remain antibiotics (vancomycin, teicoplanin, nitrofurantoin, fosfomycin and linezolid) was below 10%. None isolate showed resistance to ampicillin or gentamicin. In the 91 isolates analyzed, 85.7% proved to be multidrug-resistant. In conclusion, colostrum contains multidrug-resistant *E. faecalis* and constitutes a reservoir and vehicle for the transmission of these bacteria. For this reason, more prudent use of antibiotics in the therapy and prophylaxis of cattle is recommended, as well as, the correct management of the colostrum.

Keywords: *Enterococcus faecalis;* colostrum; dairy calves; antibiotic resistance; multidrug resistance.

Citation: Cunha, S.; Soares, R.; Maia, M.; Gilberto, I.; Silva, F.; Miranda, C.; Poeta, P. Presence of antibiotic-resistant *Enterococcus Faecalis* in colostrum supplied to calves? *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Enterococcus faecalis is a commensal bacterium and one of the main indicators of fecal contamination [1]. In immunocompromised individuals, they can cause opportunistic infections, being, therefore, one of the main causes of nosocomial infections [2,3]. This species has natural and acquired resistance to numerous classes of antibiotics, which limits

therapeutic options [4]. Resistance genes are often housed in mobile genetic elements, such as plasmids and transposons, involved in the horizontal gene transfer to other bacteria, namely pathogenic bacteria [1].

Colostrum, used in the feeding of calves, is composed of a microbiota that colonizes the animals' gastrointestinal tract early in their life [5,6]. It can be responsible for colonizing calves by antibiotic-resistant *E. feacalis*, contributing to the spread of antibiotic-resistant bacteria and antibiotic-resistant genes [1,6].

These microorganisms can then be transmitted to humans through contact with contaminated animals, or through the consumption of food from these animals [5,6]. *E. feacalis* can also be released through the calves' feces, causing environmental contamination [7]. Thus, this public health problem must be seen in a "One Health" perspective, considering the interactions between human, animal, and environmental health [8].

In this work, the aim to characterize antibiotic resistance in *E. faecalis* isolates from colostrum used in the feeding of calves.

2. Materials and Methods

2.1. Isolates

Nineteen one *Enterococcus faecalis* isolates obtained from 40 samples collected in 12 dairy farms in the Portuguese region of Entre Douro e Minho, were recovered using agar selective plates, such as Slanetz-Bartley agar and Kanamycin aesculin azide agar, and incubated at 37ºC for 24h. The identification of the isolates was confirmed by routine biochemical methods.

2.2. Antimicrobial resistance

The antimicrobial susceptibility testing was performed using antimicrobial agents by the disk diffusion method, according to the Clinical and Laboratory Standards Institute standards [9]. A total of 14 antimicrobial agents were used: quinupristin-dalfopristin (15 μg), tetracycline (30 μg), erythromycin (15 μg), streptomycin (300 μg), rifampicin (5 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), vancomycin (30 μg), linezolid (30 μg), fosfomycin (200 μg), nitrofurantoin (300 μg), teicoplanin (30 μg), ampicilin (10 μg) and gentamicin (120 μg).

3. Results

From 91 isolates, the majority showed antibiotic-resistance to tetracycline (79.1%), erythromycin (79.1%) and streptomycin (57.1%). This was followed by rifampicin (47.3%), chloramphenicol (25.3%) and ciprofloxacin (11.0%). Resistance to the remain antibiotics was below 10%: 5.5% showed resistance to vancomycin; 4.4% showed resistance to linezolid; 3.3% showed resistance to fosfomycin; 2.2% showed resistance to nitrofurantoin and 1.1% showed resistance to teicoplanin. None isolate showed resistance to ampicillin or gentamicin. In addition, all *E. faecalis* isolates were intrinsically resistant to quinupristin-dalfopristin (Table 1).

In the isolates analyzed, 85.7% proved to be multidrug-resistant (≥3 antimicrobial classes): 1.1 % showed resistance to 8 antibiotic classes, 2.2% showed resistance to 7 antibiotic classes, 5.5% showed resistance to 6 antibiotic classes, 25.3% showed resistance to 5 antibiotic classes, 35.1% showed resistance to 4 antibiotic classes and 16.5% showed resistance to 3 antibiotic classes. In contrast, 14.3% of the isolates didn't present multidrug-resistance: 8.8% showed resistance to 2 antibiotic classes and 5.5% showed resistance to 1 antibiotic class (Figure 1).

Table 1. Antibiotic resistance detected in *Enterococcus faecalis* isolates analyzed in this study.

Antibiotics	Resistant Isolates	
	Nr.	%
Quinupristin-dalfopristin	91	100*
Tetracycline	72	79.1
Erythromycin	72	79.1
Streptomycin	52	57.1
Rifampicin	43	47.3
Chloramphenicol	23	25.3
Ciprofloxacin	10	11.0
Vancomycin	5	5.5
Linezolid	4	4.4
Fosfomycin	3	3.3
Nitrofurantoin	2	2.2
Teicoplanin	1	1.1
Ampicillin	0	0
Gentamicin	0	0

*: intrinsic resistance.

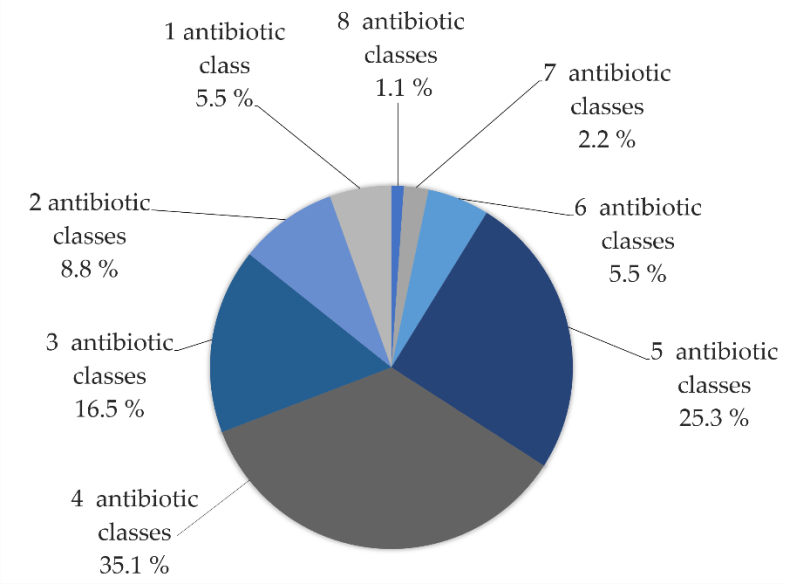

Figure 1. Percentage of *E. faecalis* isolates resistant to different antibiotic classes analyzed in this study.

4. Discussion

With this study, the analyzed colostrum that was used for feeding calves demonstrated the presence of multidrug-resistant *E. faecalis* isolates. In concordance with our results, Różańska *et al.* [10] reported the presence of *E. feacalis* isolated from mastitis milk, in which 4.0% of the isolates showed resistance to linezolid, 4.9% to nitrofurantoin, 84.6 % to quinupristin-dalfopristin, 47.7% to streptomycin, 82.0% to tetracycline and 0.9% to vancomycin.

Kumar *et al.* [12] reported results of 100% sensibility to tetracycline of *E. feacalis* isolates from raw buffalo milk. In opposite with our results, the same authors reported that all isolates showed resistance to rifampicin. From goat milk, Perin *et al.* [13] reported that all isolates of *Enterococcus* spp. showed sensitivity to ampicillin, which agrees with the

present study. However, regarding chloramphenicol, for which they report 100% sensitivity, there is no similarity between the results.

Citak *et al.* [14] revealed a percentage of resistance to erythromycin identical to that we found in colostrum: 92% of *E. feacalis* isolates from raw milk were resistant to this antibiotic. However, compared to our results, they showed a much higher percentage of isolates resistance to teicoplanin (52%) and gentamicin (63%). In another study, using raw cow's milk samples, the percentage of *Enterococcus* spp. with resistance to fosfomycin was 11.8%, slightly higher than that obtained in *E. faecalis* from colostrum. Relatively to ciprofloxacin, the percentage of resistant isolates (47.1%) was much higher than that found in colostrum [15]. Bouymajane *et al.* [15] revealed that 100% of the analyzed isolates of *Enterococcus* spp. were resistant to at least one antibiotic. Of these, 82.3% (belonging to the species *E. feacalis* and *E. faecium*) were multidrug-resistant, similar to our results.

4. Conclusions

This study showed that the colostrum contains multidrug-resistant *E. faecalis* and can constitute a reservoir and vehicle for the transmission of these bacteria. For this reason, more prudent use of antibiotics in the therapy and prophylaxis of cattle is recommended, as well as, the correct management of the colostrum.

Author Contributions: Conceptualization, S.C. and C.M.; methodology, S.C., R.S. and M.M.; validation, G.I., F.S., C.M. and P.P.; writing—original draft preparation, S.C.; supervision, G.I., F.S., C.M. and P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the R&D Project CAREBIO2 (Comparative assessment of antimicrobial resistance in environmental biofilms through proteomics - towards innovative theragnostic biomarkers), with reference NORTE-01-0145-FEDER-030101 and PTDC/SAU-INF/30101/2017, financed by the European Regional Development Fund (ERDF) through the Northern Regional Operational Program (NORTE 2020) and the Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

Conflicts of Interest: The authors declare no conflict of interest.

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