SHEDDING PATTERNS OF ANTIMICROBIAL-RESISTANT ESCHERICHIA COLI STRAINS IN A COHORT OF CALVES AND THEIR DAMS ON A BRAZILIAN DAIRY FARM

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MODELO DE EXCREÇÃO DE CEPAS DE ESCHERICHIA COLI RESISTENTES A ANTIMICROBIANOS EM UMA COORTE DE BEZERROS E SUAS RESPECTIVAS MÃES EM UMA FAZENDA DE LEITE BRASILEIRA

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SUMMARY

Rectal fecal samples were collected three times over an eight-month period from five pars of calves and their dams at the same farm, and screened for Escherichia coli resistance to eleven antimicrobial agents. An increase in the number of antimicrobial-resistant isolates was documented in the third collection either in calves and dams, though the increase was higher among calves. A statistically significant increase in the antimicrobial resistance was detected to tetracycline among the calves, and to amoxicillin+clavulanic acid and amikacin among the dams. Careful follow-up of the shedding of E. coli resistant isolates in feces is important, because the use of fresh bovine feces (manure) in land application is usual in Brazil, what could increase the risk of dissemination of antibiotic resistant genes in the environment.

KEY-WORDS: Escherichia coli. Antibiotic resistance. Calve. Dams.

RESUMO

Amostras de fezes foram coletadas de cinco pares vaca-bezerro provenientes da mesma fazenda em três ocasiões distintas em um período de oito meses, as cepas isoladas de *Escherichia coli* foram analisadas frente a onze antibióticos. Um aumento do número de cepas resistentes a agentes antimicrobianos foi detectada na terceira coleta, tanto entre os bezerros como entre as suas mães, mas este aumento foi maior entre os bezerros. Um aumento estatisticamente significante para resistência antimicrobiana foi detectado para a tetraciclina entre os bezerros e para amoxicilina+ ácido clavulânico e amikacina entre as vacas. Uma análise seqüencial cuidadosa das cepas de *E. coli* excretadas nas fezes é importante, isto porque no Brasil é comum a utilização de fezes bovinas frescas (esterco) para a utilização como adubo em plantações, o que pode aumentar o risco de disseminação de genes de resistência a antibióticos no meio ambiente.

PALAVRAS-CHAVE: *Escherichia coli*. Resistência a antibióticos. Bezerros. Vacas.

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Exposure to antibiotics increases the level of bacterial resistance of the normal commensal microbiota in both animals and humans. Bearing of resistance by commensals, in special *Escherichia coli,* has been proposed as an indicator of antibiotic resistance in a population (VAN DEN BOGAARD & STOBBERINGH, 2000). The acquisition of resistance by commensal bacteria has given rise to serious concern, because the intestinal microbiota acts as a potential reservoir of antibiotic resistance genes which may be transferred to pathogenic bacteria to hosts (SUMMERS, 2002).

Current strategies to monitor the presence of antibiotic-resistant bacteria in food animals target mainly resistance in clinical specimens and involve only periodic cross-sectional evaluations of resistance in faecal microbiota on a larger scale (CAPRIOLI et al, 2000, GUN & LOW, 2003). However, such surveys do not provide any information about the dynamic of antibiotic resistant strains in the normal microbiota.

With the increase in dairy size and cow concentration, shedding of bacteria with antibiotic-resistant genes has become more concern. Contamination of crops by cow manure used as fertilizer has become an important food safety and environmental issue (AARESTRUP et al, 1998, TEUBER, 2001).

Housing and dietary changes such as weaning may affect the prevalence of resistance by altering calf exposure to colonizing strains or changing the *E. coli* composition of the gut flora (OVERLAND et al, 2000). The principal objective of this study was to investigate the onset and subsequent pattern of shedding of antimicrobial-resistant *E. coli* in a cohort of Brazilian dairy calves over a 8-month period. The study was performed at a dairy farm (Mococa city, São Paulo State) during November 2005 to June 2006. In order to study the antimicrobial susceptibilities of *E. coli* isolates to 11 antimicrobial agents, ten animals, five pairs calf-dam were examined three times during the period above mentioned. Individual rectal faecal samples were collected by swabbing with a sterile cotton swab. Samples were placed in Stuart transport medium and taken to a laboratory for immediate processing. The samples were transferred to MacConkey Agar (Difco, Detroit,MI, USA) and incubated for 24 h at 37 ^{o}C . At least five colonies with characteristics of *E. coli* from each plate inoculated with rectal swab from calf or dam were selected for analysis. Biochemical confirmation of the strains was performed and *E. coli* was defined as oxidase negative, indole positive, Simon's citrate negative, urease negative and hydrogen sulfide negative (KONEMAN et al., 1997).

Antimicrobial disk susceptibility tests were performed using the disk diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2002). Drug-impregnated disks (CEFAR, São Paulo, BR) were placed on the surface of the agar using a disk dispenser. The following eleven antimicrobial agents were tested: ampicillin (AMP,10μg); amoxicillin (AMO,10μg); amikacin (AMK,30μg); cephalothin (CFL,30μg); ceftriaxone (CEF,30μg); gentamicin (GEN,5μg); tetracycline (TET, 30μg); streptomycin (STR,10μg); nalidixic acid (NAL,30μg); cotrimoxazole (SUT, 25μg); ciprofloxacin (CIP,5μg).

The results presented here are results for one cohort of calves and dams on a single farm and should therefore be interpreted with some caution. In each sampling period at least 50 *E. coli* isolates were examined from calves and their dams. Between the first and second period, we did not find any alteration in the pattern of shedding involving both, the calves and dams (results not shown) However, in the third collection a significant increase in the number of resistant strains were detected in both, calves and dams (Table 1) with a marked increasing among the calves. Statistically significant increase in the antimicrobial resistance was detected to tetracycline among the calves, and to amoxicillin + clavulanic acid and amikacin among the dams (Table 1). The main difference between both periods was the weaning of the calves and the modification to a grazing diet. Hinton et al (1984) reported that a sensitive flora of *E. coli* gradually emerged following weaning although calves that had been exposed to medicated feed did not develop a fully sensitive gut flora post-weaning during the first weeks. Dietary changes such as weaning may affect the prevalence of resistance by altering calf exposure to colonizing strains or changing the *E. coli* composition of the gut flora (OVERLAND et al, 2000, HOYLE et al, 2004). Khachatryan et al (2004) speculated that one reason for the young animals shed more resistant organisms in that this cohort may be exposed to greater amounts of antimicrobial drugs for medication. In addition the intestinal physiology of younger animals is different from that in older animals and thus there may be niche specific clones that are better suited to the calf intestinal environment.

Overland et al (2000) reported that the carriage of resistant bacteria was associated with both age and housing status of the cohort. Hoyle et al (2004) verified a shedding peak to ampicillin resistance among the *E. coli* strains between the 2 -10 week of sampling and presented another hypothesis, that after housing the altered exposure to other animals and colonizing strains on the farmstead had a greater impact than the diet in the intestinal tract microbiota..

Pearce et al (2004) reported a amazing fact with a cohort of calves, that the shedding of *E. coli* with the specific serogroup O26 by calves, began within a few weeks of birth and there was a marked pulse of shedding during the weeks 3 to 7, when the prevalence of shedding was above 30% and peaked at 60%, after this time, the prevalence of shedding dropped abruptly and remained much lower. The authors did not present a conclusively explanation for this effect but suggest that environment may have an influence on the shedding of *E. coli* in calves. Houser et al (2008) showed that the effects of environmental perturbations (diet, antibiotics, or pathogens) on the normal gut microbiota can have a strong influence on the clonal types that are present at a given time of sampling. The possibility of calves acting as an antimicrobial resistant genes reservoir, with horizontal transmission to other farm livestock is a real possibility, so the management of the calves or their products (carcasses, meat, manure, etc) would require extra care to minimize these risks.

The carefully accompanying of the shedding of *E. coli* resistant isolates in feces is important because in Brazil is usual to use fresh bovine feces (manure) in land application, what could increase the risk of dissemination of antibiotic-resistant genes in the environment, so it is important to apply the correct treatment to minimize the risk by doing a composting system. To conclude, the present study showed that the calf after weaning eliminate a more dangerous organisms with high level of antibiotic resistance what should be carefully treated in composting systems. Further cohort studies are required to ascertain whether the results obtained here are cohort specific or reflect a general pattern of *E. coli* shedding in calves.

Table 1 – Antimicrobial susceptibility testing of *Escherichia coli* strains isolated from feces of five pairs of healthy cows and calves in the first (November / 2005) and third (July / 2006) sample collection.

Antimicrobial agents	**Number of resistant strains (%) 1º collection**	**Number of resistant strains (%) 3º collection**	***P* value****
	Cows (22 isolates)	**Cows** (25 isolates)	
Gentamicin	- *	-	-
Ciprofloxacin	-	-	-
Ceftriaxone	-	1 (4.0)	1.0000
Amoxicillin + clavulanic acid	-	5 (20.0)	0.0002
Cotrimoxazole	4 (18.0)	2 (8.0)	0.3980
Nalidixic acid	1 (4.5)	5 (20.0)	0.1936
Amikacin	-	5 (20.0)	0.0002
Ampicillin	2 (9.0)	3 (12.0)	1.0000
Tetracycline	6 (27.0)	10 (40.0)	0.5381
Streptomycin	2 (9.0)	3 (12.0)	1.0000
Cephalothin	-	3 (12.0)	0.2368
	Calf (21 isolates)	**Calf** (25 isolates)	
Gentamicin	-	3 (12.0)	0.2391
Ciprofloxacin	-	2 (8.0)	0.4928
Ceftriaxone	-	1 (4.0)	1.0000
Amoxicillin + clavulanic acid	1 (4.7)	5 (20.0)	0.1981
Cotrimoxazole	-	4 (16.0)	0.1142
Nalidixic acid	3 (14.2)	8 (32.0)	0.1878
Amikacin	4 (19.0)	10 (40.0)	0.1988
Ampicillin tetracycline	1 (4.7)	3 (12.0)	0.6139
Tetracycline	1 (4.7)	9 (36.0)	0.0132
Streptomycin	2 (9.5)	5 (20.0)	0.4285
Cephalothin	2 (9.5)	7 (28.0)	0.1506

* not found
**Fisher's exact test, statistically significant p< 0. 05

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