

## Short Communication

# Prevalence and antibiotic resistance of mastitis pathogens isolated from dairy herds transitioning to organic management

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**Changes in udder health and antibiotic resistance of mastitis pathogens isolated from dairies upon conversion from conventional to organic management over a 3-year period was studied. Coagulase-negative staphylococci (CNS) were the most prevalent mastitis pathogens isolated. CNS were significantly less resistant to  $\beta$ -lactam antibiotics when isolated from milk after the herd transitioned to organic management. Cessation of the use of antimicrobial therapies in dairies in combination with organic management could lead to a reduction in the antimicrobial resistance of mastitis pathogens.**

**Keywords:** antibiotic resistance, mastitis pathogens, organic dairy

In USA, organic dairy producers must follow strict guidelines prohibiting the use of antibiotics to animals that are considered to be organic [9]. Conventionally managed dairy herds proceed through a full year of transition before being certified organic and during this transition year must follow all guidelines required of organic dairy herds. Reduced use of antibiotics is related with a reduction in the amount of antimicrobial-resistant bacteria [4,7]. Lowering the number of pathogens resistant to antibiotics is a global concern in the animal livestock industry [1]. The purpose of this study was to determine changes in intramammary infection (IMI) prevalence and antimicrobial resistance in mastitis pathogens isolated at the end of lactation and at parturition. Two herds in their last year of conventional dairy production, during their transition year, and during their first year of organic production, were studied. During the conventional period, cows with clinical mastitis

received a commercial lactating cow intramammary product with pirlimycin hydrochloride (Pfizer Animal Health, USA). Non-lactating cow intramammary products of cephapirin sodium or dihydrostreptomycin sulfate and penicillin G procaine or novobiocin sodium and penicillin G procaine (Pfizer Animal Health, USA) were used. Composite milk samples were aseptically collected from cows at the end of lactation and within 1 day of parturition, and from cows with clinical mastitis, during all three years. Milk was cultured on blood agar plates and mastitis pathogens identified [6] as summarized in Table 1. IMI was determined using guidelines as described [2]. Antibiotic resistance testing was as described [2]. Interpretation of zone diameter was carried out according to Clinical and Laboratory Standard Institute (CLSI) guidelines [10]. Some isolates lost viability during storage. Five and 14 coagulase-negative Staphylococci (CNS) isolates from cows in the transition and organic periods, respectively, were not recoverable after storage. Statistical analysis included: Mantel-Haenszel Chi-Square analysis, Wilcoxin Rank Sum test and the Kruskal-Wallis test for pair-wise comparisons (ver. 8.0; SAS, USA).

At parturition, there was a decrease in the percentage of cows free of IMI as herds transitioned to the organic management period compared to the conventional period ( $p = 0.006$ , Table 1). However, the percentage of non-infected cows at dry-off was not significantly different ( $p = 0.445$ , Table 1). The most prevalent pathogen type that caused IMI was CNS, followed by *Streptococcus* spp. other than *Streptococcus agalactiae* at parturition and dry-off (Table 1). The prevalence of *Staphylococcus* (*S. aureus*) IMI did not change significantly as herds transitioned from conventional management to organic certification

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**Table 1.** The number of cows with intramammary infection (IMI) and free of IMI at parturition and dry-off in herds certified as organic and herds transitioned from conventional management

Status	Period	Parturition			Dry-off		
		Conventional (1st year)	Transition (2nd year)	Organic (3rd year)	Conventional (1st year)	Transition (2nd year)	Organic (3rd year)
Detected pathogens	Infection cases	39 (47.0)	63 (61.8)	74 (69.8)	31 (44.9)	28 (40)	22 (42.3)
	Coagulase-negative staphylococci	27 (32.5)	36 (35.3)	47 (44.3)	21 (30.4)	20 (28.6)	19 (36.5)
	<i>Staphylococcus aureus</i>	6 (7.2)	7 (6.9)	4 (3.8)	5 (7.2)	3 (4.3)	1 (1.9)
	<i>Streptococcus</i> spp.	6 (7.2)	16 (15.7)	21 (19.8)	5 (7.2)	4 (5.7)	1 (1.9)
	Coliforms	0	3 (2.9)	2 (1.9)	0	0	1 (1.9)
	Other	0	1 (1.0)	0	0	1 (1.4)	0
	Non-infection cases	44 (53.0)	39 (38.2)	32 (30.2)	38 (55.1)	42 (60.0)	30 (42.3)
	Total	83	102	106	69	70	52

Values are number (%).

**Table 2.** Inhibition zone diameter of mastitis pathogens by period

Pathogens	Period	Antibiotics											
		Amp	Ceph	Clox	Ery	Gen	Lin	Neo	Nitr	Nov	Pen	Strep	Tet
Coagulase-negative staphylococci	Conventional	28.7	36.9	17.3	25.2	27.2	16.7	25.4	22.5	27.9	29.6	18.6	29.6
	Transition	30.7	37.9	17.3	27.1	27.4	18.3	25.9	21.8	28.9	32.4	18.9	30.0
	Organic	34.3*	39.2 <sup>†</sup>	19.9 <sup>‡</sup>	27.1	27.7	17.6	26.1	22.1	29.3	36.1 <sup>§</sup>	19.7	30.3
<i>Staphylococcus aureus</i>	Conventional	26.9	33.5	19.7	25.6	23.5	20.2	23.2	20.7	29.8	28.2	16.9	28.3
	Transition	23.5	29.7	17.8	24.3	21.2	18.7	21.4	17.8	28.0	24.8	15.5	25.5
	Organic	25.0	32.2	20.0	24.2	22	15.6	21.6	17.8	26.8	25.8	15.6	23.2
<i>Streptococcus</i> spp.	Conventional	35.0	39.0	26.5	32.5	22.3	17.6	23.7	24.9	19.1	39.3	16.5	26.1
	Transition	37.0	36.8	27.0	29.8	22.6	19.0	20.6	26.5	16.1	37.5	16.8	18.0
	Organic	36.8	37.2	25.2	28.2	20.6	17.3	19.2	25.8	17.1	38.8	13.1	20.6

Amp: ampicillin, Ceph: cephalothin, Clox: cloxacillin, Ery: erythromycin, Gen: gentamicin, Lin: lincomycin, Neo: neomycin, Nitr: nitrofurantoin, Nov: novobiocin, Pen: penicillin, Strep: streptomycin, Tet: tetracycline, Unit: mm. \* $p = 0.0034$ , <sup>†</sup> $p = 0.0214$ , <sup>‡</sup> $p = 0.0053$ , <sup>§</sup> $p = 0.0024$ .

(Table 1). Only six cases of coliform mastitis were detected during the 3-year study, and two of them were isolated from clinical mastitis. There was a significant increase in zone diameter around the ampicillin, cephalothin, cloxacillin, and penicillin discs for CNS according to the period (conventional vs. transition vs. organic phase) ( $p < 0.05$ , Table 2). However, there were no significant changes in the sensitivity patterns of the antibiotics to *Streptococcus* spp. and *S. aureus* (Table 2). Antibiotic resistance of the coliforms was not analyzed due to the low infection rate. The percentage of CNS isolates deemed resistant to  $\beta$ -lactam antibiotics decreased from the conventional period compared to the organic period.

The percentage of cows displaying IMI at parturition was significantly greater during the transition (61.8%) and

organic periods (69.8%) than that during the conventional period (47.0%) (Table 1). This increase in IMI during the transition and organic periods was attributed to the cessation of dry cow antibiotic therapy as such therapy reduces the frequency of IMI at parturition [3]. This means that IMI at parturition has a tendency to be spontaneously cured during lactation, consistent with a report [8]. However, the frequency of IMI at dry-off did not differ between the study periods (conventional, transition, and organic; Table 1). In this study, dry cow antibiotic therapy in the conventional period was not significantly effective in lowering the IMI rate compared to organic management during the dry period. Only at parturition, antibiotic therapy seemed to be effective in lowering the IMI rate from the conventional to organic periods. A significant

change in  $\beta$ -lactam mean zone diameters was noted for the CNS isolates in the organic period compared to those in the conventional and transition periods (Table 2). On the other hand, changes in the zone diameters for *S. aureus* and *Streptococcus* spp. were not significant. The isolation rate of *S. aureus* and *Streptococcus* spp. was relatively small, and the variation was large. Moreover, the percentage of CNS isolates deemed resistant to  $\beta$ -lactam antibiotics was highest during the conventional as opposed to organic period. Studies [4,5,7] have reported that CNS strains isolated from organic herds are less resistant than those from conventional herds. As  $\beta$ -lactams are historically the primary group of antibiotics used in both dairies, cessation of  $\beta$ -lactam use should lead to the establishment of pathogen clones that are less resistant to these antibiotics.

In conclusion, this is the first study documenting a change in antibiotic resistance that occurs during the longitudinal transition from conventional to organic management. In this study, the decreased  $\beta$ -lactam resistance rate of CNS paralleled the discontinuation of the use of  $\beta$ -lactam antibiotics. The aggregate results of this longitudinal study as well as other cohort studies comparing cows managed conventionally vs. organically suggest that cessation of the use of antibiotics in a dairy farm transitioning to organic management would decrease the number of antibiotic-resistant pathogens.

## References

1. **Aarestrup FM.** Monitoring of antimicrobial resistance among food animals: principles and limitations. *J Vet Med B Infect Dis Vet Public Health* 2004, **51**, 380-388.
2. **Barnes-Pallesen FD, Blackmer P, Britten A, Bushnell RB, Van Damme DM, Welcome F.** Laboratory and Field Handbook on Bovine Mastitis. 1st ed. pp. 151-175, National Mastitis Council, Arlington, 1987.
3. **Berry EA, Hillerton JE.** The effect of selective dry cow treatment on new intramammary infections. *J Dairy Sci* 2002, **85**, 112-121.
4. **Bombyk RA, Bykowski AL, Draper CE, Savelkoul EJ, Sullivan LR, Wyckoff TJ.** Comparison of types and antimicrobial susceptibility of *Staphylococcus* from conventional and organic dairies in west-central Minnesota, USA. *J Appl Microbiol* 2008, **104**, 1726-1731.
5. **Garmo RT, Waage S, Sviland S, Henriksen BI, Osteras O, Reksen O.** Reproductive performance, udder health, and antibiotic resistance in mastitis bacteria isolated from Norwegian Red cows in conventional and organic farming. *Acta Vet Scand* 2010, **52**, 11.
6. **Hogan JS, González RN, Harmon RJ, Nickerson SC, Oliver SP, Pankey JW, Smith KL.** Laboratory Handbook on Bovine Mastitis. Revised ed. pp. 41-151, National Mastitis Council, Wisconsin, 1999.
7. **Pol M, Ruegg PL.** Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. *J Dairy Sci* 2007, **90**, 249-261.
8. **Pyörälä S, Taponen S.** Coagulase-negative staphylococci-emerging mastitis pathogens. *Vet Microbiol* 2009, **134**, 3-8.
9. **Ruegg PL.** Management of mastitis on organic and conventional dairy farms. *J Anim Sci* 2009, **87** (Suppl 1), 43-55.
10. **Wikler MA, Cockerill FR 3rd, Craig WA, Dudley MN, Eliopoulos GM, Hecht DW, Hindler JF, Low DE, Sheehan DJ, Tenover FC, Turnudge JD, Weinstein MP, Zimmer BL, Ferraro MJ, Swenson JM.** Performance Standards for Antimicrobial Susceptibility Testing. Vol. 26. pp. 44-51, Clinical and Laboratory Standards Institute, Wayne, 2006.