



## Milk Quality and Antimicrobial Resistance against Mastitis Pathogens after Changing from a Conventional to an Experimentally Organic Dairy Farm

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**ABSTRACT :** The present study was to investigate the effect of the transition from conventional to organic dairy farming on the antimicrobial resistant pattern of pathogens in milk. A farm with tie-stall management, with an average herd size of 20 milking cows, was selected based on the owner's willingness to accept, for at least 6 months, the highly restricted protocol developed in this study. Comparisons of bacterial isolates and antimicrobial susceptibilities before changing to an organic farm system (BEFORE) and for 6 months after (AFTER) operating the experimental organic farm system were performed by Fisher's Exact Chi-square tests. Significant levels were defined at  $p < 0.05$ . During the AFTER period, average frequency of antibiotic treatment was decreased from more than 3 cases/month to less than 1 case/month during which the antibiotic use was authorized only by the veterinarian. In total, 92 and 70 quarter milk samples from 24 and 18 cows during BEFORE and AFTER, respectively, were included in the study. Overall, isolates ranged from a non-resistant level for cephalosporin to a very high resistant level to streptomycin (64.71% to 95.45%). Percentages of antimicrobial resistant isolates during BEFORE were significantly higher than during AFTER for ampicillin (43.48% and 5.88%, respectively) and streptomycin (95.45% and 64.71%, respectively). In conclusion, percentages of antimicrobial resistant isolates were decreased after 6 months of operating as an organic farm system. (**Key Words :** Organic Dairy Farm, Antibiotic Resistant, Mastitis Pathogens, Cattle)

### INTRODUCTION

Organic food has been accepted by consumers concerned about their health, for its quality. In addition, the popularity of natural and organic foods is increasing as many consumers seek what they perceive as safer and more nutritious food produced under sustainable and ethical conditions, especially as regards animal welfare. The principles of organic livestock production have been formulated by the International Federation of Organic Agriculture Movements (IFOAM) and were included in EU regulation 2092/91 in the year 2000. Organic farming is based on principles which explicitly state that animal welfare is a high priority (Lund, 2006). "Organic foods" in the USA have been defined in the Code of Federal Regulations (7CFR205) with standards set as to which processes and synthetic substances are acceptable for production and handling in order for foods to be certified as organic. Thus "organic" is a production claim and not a food safety claim. In the UK, organic farming standards include the feeding of a minimum of 60% of the dry matter

intake as forage, and aim to provide a basis for optimal dairy cow comfort and health (Anon, 2005). Recent years have seen an increase in the number of dairy farms that have converted to organic farming. Organically farmed cows are fed with foodstuffs grown without pesticides and are subject to different EU regulations on housing standards and the use of veterinary medicines (CEC, 2004). The principles behind organic production and many organic producer groups suggest that more attention is paid to animal welfare on organic farms than on non-organic farms (Sundrum, 2001).

Concerns have been raised that by not using the appropriate synthetic medicine to treat disease, cows may be suffering prolonged exposure to diseases, thereby reducing their welfare (Hovi et al., 2003). In Europe, bovine mastitis is recognized as the major contributor to economic loss on dairy farms, and it continues to be routinely treated with antibiotics on organic farms (Biggs, 1999). EU regulations governing the frequency of antibiotic treatments allow individual cows to be treated up to three times during lactation without prejudicing their organic status (CEC, 2004). Increased milk withdrawal periods provide a disincentive to such treatment.

The emergence of multidrug resistant pathogens has

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accelerated in the past 10 to 15 years (Shea, 2003). The use of antibiotics in animal husbandry is a driving force for the increase of antimicrobial resistance in certain pathogenic bacterial species (Bates et al., 1994; Witte, 1998). Some bacteria are resistant to most antimicrobial agents, which provide a growing problem in humans and in veterinary medicine (Levy, 1998). The public health problem is the potential impact of the transmission of resistant bacteria to humans via the food chain, as in bulk milk with subclinical mastitis. In veterinary medicine, there has been a reduction in cure rates after treatment of bacterial diseases, especially clinical mastitis cases (Owens et al., 1997; Sol et al., 2000). The World Health Organization (WHO) has stated that any use of antimicrobial agents is associated with the risk of inducing resistance to antimicrobial agents among bacteria (WHO, 1997). The existence of antimicrobial resistance of udder pathogens is worldwide as is shown by recent studies in New Zealand and Denmark (Salmon et al., 1998), the United States (Rossitto et al., 2002), and Finland (Pitkä lä et al., 2004).

Sato et al. (2004a) found small differences on antimicrobial resistance between organic farms and conventional farms in the United States and Denmark. Furthermore, some studies with dairy cattle have reported more antimicrobial resistant bacteria from conventionally raised animals (Sato et al., 2004a; Halbert et al., 2006; Ray et al., 2006). Lower prevalence of antimicrobial resistance of mastitis pathogens such as *Staphylococcus aureus* strains was found on organic farms than on conventional farms in the United States (Tikofsky et al., 2003). In contrast, others reported similar incidences of resistance in bacteria from animals in both raising systems (Sato et al., 2004b; Roesch et al., 2006). However, most studies compared farms that have been established as either organic or conventional for a few years. The aim of the present study was to investigate the effects of antimicrobial resistant pattern of pathogens in bovine milk during the transition from conventional to organic farming.

## MATERIALS AND METHODS

### Farm selection and milk sample collection

A farm with tie-stall management with an average herd size of 20 milking cows from the Mae-On Dairy Cooperative, participating in the herd health management program of the Faculty of Veterinary Medicine, Chiang Mai University, was selected, based on the owner's willingness to comply with the highly restrictive transition protocol developed in this study for at least 6 months. The study was carried out from August 07 to March 08. The farm had calving all year around, with average days in milk in August 07 and March 08 being 152.1 and 162.6 days respectively. Most cows were fed post-harvest corn stem and straw *ad lib*,

and concentrates according to their milk production. The majority of the cows were crossbred Holstein-Friesian. Before the study, the farm was managed as a conventional farm, including tie-stall management, feeding with local agricultural product, urea treated straw during dry period, freely using veterinary drugs including hormones for either treatment or prevention, and synthetic pesticides on a routine basis.

All milking cows were selected and their milk samples separated on quarter samples were collected at before changing to organic farm system (BEFORE) and at the end of study or 6-months after operating as organic farm system (AFTER). Milk samples were collected with aseptic techniques in accordance with National Mastitis Council guidelines (NMC, 1999). Briefly, an udder and teat were cleaned with disinfectant. The teat ends were scrubbed with a cotton ball moistened with 70% ethanol solution until they were no longer visibly dirty. The couple streams of milk were discarded before the samples were collected into sterile test tubes. The samples were kept in cool temperature and were transported to the laboratory immediately for bacterial identification. The data on owner, cow identification, quarter, sampling and date of sampling were recorded.

### Experimental organic dairy farm protocol

The experimental organic dairy farm protocol developed in this study was based on the protocol of organic farms in EU and USA. The farmer maintained records sufficient to preserve the identity of all organically managed animals and edible and non-edible animal products produced on the operation. The farmer provided livestock with feed composed of agricultural products, including pasture and forage. At least 80% of the whole roughage during experimental period was known to have been organically produced. The farm was not allowed to use animal drugs, including hormones, to promote growth, to provide feed supplement or additives in amounts above those needed for adequate nutrition and health maintenance, to use feed formulas containing urea and manure, or to feed mammalian or poultry slaughter by-products. In livestock health care, the farmer established and maintained preventive livestock health care practices including provision of a feed ration sufficient to meet nutritional requirement, establishment of appropriate housing, pasture conditions, and sanitation practices to minimize the occurrence and spread of diseases and parasites, provision of conditions which allow for exercise, freedom of movement, and reduction of stress appropriate to dairy cattle. The farmer did not administer any animal drug, other than vaccinations, in the absence of illness, nor hormones for growth promotion, nor synthetic pesticides on a routine basis. The cows were kept in living condition to maximise

health and allow them to behave naturally including access to outdoor and to pasture with appropriate standard of cleanliness.

### Bacterial identification

Microbiological examination was performed according to the standards described in the National Mastitis Council's guideline (NMC, 1999). Ten microliters of an individual quarter milk sample was cultured on 5% bovine blood agar plates and MacConkey agar plates. Plates were incubated at 37°C for 24-48 hours. Bacterial colonies were identified based on gross morphology, a number of colonies and the hemolytic pattern. Appropriate tests were performed on the colonies isolated to identify the pathogens, including Gram staining and a catalase test to classify streptococci and staphylococci genera. The hemolytic patterns and coagulase reaction with rabbit plasma were used to differentiate between *Staphylococcus aureus* and coagulase negative staphylococci (CNS), and the esculin hydrolysis and CAMP reaction were used to identify *Streptococcus agalactiae* and environmental streptococci. *Corynebacterium bovis* and *Arcanobacterium pyogenes* were identified using culture characteristic on blood agar, motility and catalase reaction test. Gram-negative bacteria were identified to Enterococci spp. using culture morphology on MacConkey agar (Merk, Germany), lactose fermentation, motility and reaction in triple sugar iron. Other colony types were grouped as other microorganisms. Degree of confidence in diagnosing an infection was classified as either not significant, questionably significant, probably significant and highly significant, based on the National Mastitis Council's guidelines (NMC guideline). Samples that contained three or more bacterial species were considered to be contaminated except those sample containing either *Streptococcus agalactiae* or *Staphylococcus aureus* were always defined as intramammary infection (IMI) (NMC,

1999).

### Susceptibility testing

The highly significant isolates were subsequently tested for antimicrobial susceptibility by the agar disc diffusion method in accordance with the standard in National Mastitis Council-NMC guidelines (NMC, 1999). The entire surface of agar plates was inoculated by using sterile cotton swab. The isolates were submitted to seven antimicrobials: ampicillin (10 µg), cloxacillin (30 µg), penicillin (30 IU), gentamicin (10 µg), streptomycin (10 µg), cephalosporin (30 µg) and sulfa-trimethoprim (10 µg), and plates were incubated at 37°C for 24 h. Subsequently, the diameter of the zone of inhibition around disc was measured. The isolated microorganisms were categorized to susceptibility and resistance according to methods and criteria described by National Committee for Clinical Laboratory Standards (NCCLS, 2002).

### Statistical analysis

Data on distribution of bacterial isolates and antimicrobial susceptibility were described as frequencies and percentages. Comparisons of bacterial isolates and antimicrobial susceptibilities between BEFORE and AFTER were performed by Fisher's Exact Chi-square tests. The significant levels were defined at  $p < 0.05$ .

## RESULTS

During the 6-months operating as an organic farm, averages of a frequency of antimicrobials treatment were decreased from more than 3 cases/month to less than 1 case/month with the drug use being authorized only by the veterinarian. Frequencies of bacteria isolation from IMI quarters between BEFORE and AFTER were shown in Table 1. The overall quarter prevalence of IMI at BEFORE

**Table 1.** Bacterial isolates of quarter milk samples from all milking cows in a small holder dairy farm at before (BEFORE) and 6-month after (AFTER) operating as organic farm system in Thailand

	Operating as organic farm system					
	BEFORE (n = 92)			AFTER (n = 70)		
	n	% of isolates	Prev IMI <sup>1</sup>	N	% of isolates	Prev IMI <sup>1</sup>
<i>S. aureus</i>	1	2.00	1.09	1	4.55	1.43
CNS	23	46.00	25.00*	10	45.45	14.29*
<i>St. agalactiae</i>	0	0.00	0.00	0	0.00	0.00
Env. Streptococci	8	16.00	8.70	5	22.73	7.14
<i>A. pyogenes</i>	7	14.00	7.61*	1	4.55	1.43*
<i>C. bovis</i>	9	18.00	9.78	5	22.73	7.14
<i>E. coli</i>	0	0.00	0.00	0	0.00	0.00
Others	2	4.00	2.17	0	0.00	0.00
Total isolates	50	100.00	54.35**	22	100.00	31.43**

<sup>1</sup> Quarter prevalence of IMI was the percentage of specified isolates on the total collected samples.

\* Differences between before and after operating as organic farm at  $p < 0.1$ .

\*\* Differences between before and after operating as organic farm at  $p < 0.05$ .

(54.35%) was significantly higher than that at AFTER (31.43%). Coagulase negative staphylococci was the main bacteria isolated in this study on both BEFORE (25.00%) and AFTER (14.29%). Prevalence of IMI from CNS and *A. pyogenes* at BEFORE were higher than those at AFTER ( $p < 0.1$ ).

#### *In-vitro* antimicrobial susceptibility test

From the total of 72 isolates, only 40 strains were highly significant isolates that included for antimicrobial susceptibility test. All isolates showed 100% susceptibility to cephazolin. Both *S. aureus* isolates from BEFORE and AFTER were resistant to streptomycin, but were susceptible to all other antimicrobial agents. The results of other antimicrobial sensitivity testes overall and for the different isolates are presented in Table 2. Overall isolates ranged from a low resistant level at 0% for cephazolin to a very high resistant level ranging between 64.71% to 95.45% for streptomycin. Percentages of antimicrobial resistant at BEFORE were significantly higher than AFTER on ampicillin (5.88% and 45.45%) and streptomycin (95.45% and 64.71%). Coagulase negative staphylococci isolates were highly resistant to gentamicin (100%) and low resistant to cloxacillin (14.29%) and streptomycin (14.29%) at BEFORE. All percentages of antimicrobial resistant for CNS at BEFORE seemed higher than AFTER, with gentamicin showing significantly higher than AFTER. Environmental streptococci were highly resistant to gentamicin (87.5%-100%) and streptomycin (62.5%-100%). Most antimicrobials at BEFORE showed less resistant percentages than AFTER except ampicillin. Environmental streptococci resistance to sulfa-trimethoprim BEFORE less than AFTER ( $p < 0.05$ ). *A. pyogenes* was highly resistant to gentamicin during BEFORE (100%).

## DISCUSSION

In Thailand, organic livestock regulation is defined by

Thai agricultural commodity and food standard (TACFS 9000-2005, 2005). The changeover from conventional to organic dairy farm has to have a transitional period of at least 90 days for entering new cows from a conventional farm and a 6-month lag time before producing organic dairy products. The experimental organic farm in this study was in the transitional period of changing from conventional farm to organic farm for 6 months. The change from free use of antimicrobials by farmers to the controlled use by veterinarians in the present study allowed comparison of the antimicrobial resistance of mastitis pathogens in the BEFORE and AFTER periods. The seven antimicrobials tested are representatives of the classes of drugs used to treat mastitis in dairy farms (aminoglycosides, cephalosporins,  $\beta$ -lactams, sulfa-trimethoprim) or are groups used in human medicine (cephalosporins, gentamicin,  $\beta$ -lactams).

In total, occurrence of IMI of BEFORE (54.35%) was significantly higher than that of AFTER (31.43%). The IMI reported here was in the range of previous reports in the tropical country including India (Roman et al., 2000), Tanzania (Karimuribo et al., 2006) and Brazil (Ribeiro et al., 2009). Coagulase negative staphylococci was the most commonly isolated bacteria in this study on both BEFORE (25.00%) and AFTER (14.29%). In USA and Europe, *Staphylococcus* spp., which is not *S. aureus*, or coagulase negative staphylococci (CNS) are the predominant bacteria causing mastitis (Makovac and Ruegg, 2003; Pitkala et al., 2004). Furthermore, CNS followed by *Corynebacterium bovis* become more common isolated bacteria in the current study. This result was similar to the previous report in Finland (Pitkala et al., 2004). Many reasons for differences in pathogen distribution might be due to cow, management and environment factors (Busato et al., 2000; Roman et al., 2000; Karimuribo et al., 2006). Seasons are a factor influencing pathogen distribution in many countries. Therefore, the differences of intramammary infection prevalences between BEFORE and AFTER might be due to

**Table 2.** Percentages of antimicrobial resistant of milk samples from intramammary infected bacteria compared between before (BEFORE) and 6-month after operating on organic farm management protocol (AFTER) of a small holder dairy farm

Antimicrobial agent <sup>1</sup>	Overall		CNS		Env. Strep.		<i>A. pyogenes</i>		<i>C. bovis</i>	
	BEFORE (n = 23)	AFTER (n = 17)	BEFORE (n = 7)	AFTER (n = 6)	BEFORE (n = 8)	AFTER (n = 5)	BEFORE (n = 6)	AFTER (n = 1)	BEFORE (n = 1)	AFTER (n = 4)
AMP	43.48**	5.88**	57.14	16.67	25	0	50	0	100	0
PEN	8.70	17.65	28.57	16.67	0	20	0	0	0	25
CLX	4.35	5.88	14.29	0	0	20	0	0	0	0
GEN	26.09	29.41	100**	33.33**	87.5	100	100	0	100	75
STR	95.65**	64.71**	14.29	0	62.5	100	0	0	0	0
SFX	30.43	35.29	57.14	16.67	37.5**	100**	0	0	0	0

\*\* Differences of resistant percentages between before and after operating as organic farm at  $p < 0.05$ .

<sup>1</sup> Antimicrobial agents including 10  $\mu$ g of ampicillin (AMP), 30  $\mu$ g of cloxacillin (CLX), 30  $\mu$ g of penicillin (PEN), 10  $\mu$ g of Gentamicin (GEN), 10  $\mu$ g of streptomycin (STR) and 10  $\mu$ g of Sulfa-trimethoprim (SFX).

the seasonal differences between the 2 periods.

Overall the strains isolated from cows remained susceptible to cephalixin, as cephalosporins, in our study. Similar study in this same area showed that cephalosporins were inefficient for mastitis pathogens, especially for CNS (Boonyayatra et al., 2007). The use of cephalosporins in intramammary infusion for treatment of mastitis in cattle has been established for less than 10 years in the Thai dairy industry. At the moment, cephalosporins are not drugs of choice for dairy farmers as they are expensive. In our study, percentages of antimicrobial resistant at BEFORE were significantly higher than AFTER on ampicillin (5.88% and 45.45%) and streptomycin (95.45% and 64.71%). In accordance with the guidelines for organic farm in this study, the use of antimicrobial agents BEFORE was higher than during AFTER. This measure should lead to a lower development of antimicrobial resistance at AFTER because a close relationship was found between levels of antimicrobial resistance and the exposure to the used drugs (Lopez-Lozano et al., 2000). For environmental streptococci, most antimicrobials used in this study had high levels of resistance (ranges between 72.1 and 87.7). These results are higher than those in previous reports (Busato et al., 2000; Erskine et al., 2002). The high percentage of resistance to penicillin of this pathogen was probably related to its being the most common antimicrobial available in the market and consequently the first choice of treatment. Furthermore, widely-used sulfa-trimethoprim, and gentamicin to treat gastro-intestinal and other diseases in cattle have probably increased the multiple drug resistance of mastitis pathogens.

In conclusion, percentages of antimicrobial resistant were decreased after 6-month operating as organic farm system. This indicates that the organic dairy farm system would mitigate problems in public health and in veterinary medicine by reducing the potential impact of transmission of resistant bacteria to humans via the food chain and by increasing cure rates after treatment of bacterial diseases in dairy cattle respectively.

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