Antimicrobial susceptibility of *Escherichia coli* isolates from dairy farms using organic versus conventional production methods

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Objective—To compare antimicrobial susceptibility patterns of *Escherichia coli* isolates cultured from fecal samples from cows and calves on dairy farms that used organic (ie, no or severely limited antimicrobial use) versus conventional production methods.

Design—Cross-sectional study.

Sample Population—Fecal samples from 10 cows and 10 calves on each of 30 organic dairy farms and 30 neighboring conventional dairy farms in Wisconsin.

Procedure—*E coli* isolates obtained from the fecal samples were tested for susceptibility to 17 antimicrobials by means of a microbroth dilution test. Prevalence of antimicrobial resistance was compared between organic and conventional dairy farms.

Results—*E coli* was isolated from 1,121 (94%) fecal samples. Farm type (organic vs conventional) and animal age (cow vs calf) were significantly associated with odds that *E coli* isolates would be resistant to various antimicrobials. After controlling for age, logistic regression analyses indicated that isolates from conventional dairy farms had significantly higher rates of resistance to ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, tetracycline, and sulfamethoxazole than did isolates from organic dairy farms. However, no significant differences were detected for the 10 other antimicrobials that were tested.

Conclusions and Clinical Relevance—Results indicated that compared with isolates from conventional dairy farms, *E coli* isolates from organic dairy herds have significantly lower prevalences of resistance to 7 antimicrobials; however, prevalence of resistance was not significantly different for 10 other antimicrobials. Resistance was more common for isolates from calves than for isolates from adult dairy cows. (*J Am Vet Med Assoc* 2005;226:589–594)

The use of antimicrobials in animal agriculture is suspected to be contributing to the antimicrobial resistance problem in human medicine, with resistant bacteria isolated from some infected human patients having been traced back to farm animals.[1-5] Farm-level studies[6,7] have shown an association between antimicrobial use in food-producing animals and the prevalence of antimicrobial resistance. In general, farms with more extensive antimicrobial use have a higher proportion of resistant bacteria and a higher number of strains with multidrug resistance patterns. However, how much livestock production practices contribute to the growing antimicrobial resistance problem is unclear.[8,9]

Because milk from treated cows must be withheld from sale, antimicrobials are typically used less frequently in dairy cattle than they are in other food-producing animals.[10] In adult cows, antimicrobials are used most often for the treatment or prevention of mastitis,[11] whereas in dairy calves, antimicrobials are used most often for the treatment or prevention of diarrhea and pneumonia.[12,13]

Even given the relatively low level of antimicrobial use in conventional dairy farming, some dairy producers have elected, for various reasons, to reduce antimicrobial use even further and have adopted organic production methods under which antimicrobial use is not allowed or severely restricted. Intuitively, it would seem that eliminating antimicrobial use would decrease the prevalence of antimicrobial resistance in enteric bacteria from dairy cattle. Whether or to what extent this is true, however, is not known. Therefore, our objective was to determine whether adoption of organic production methods was associated with a decrease in the prevalence of antimicrobial resistance among enteric bacteria from dairy cattle.

In a previous study,[14] Enterobacteriaceae were isolated from human fecal samples and antimicrobial resistance patterns of *Escherichia coli* isolates were compared with resistance patterns of *Citrobacter*, *Klebsiella*, and *Proteus* isolates. The authors found a lack of antimicrobial resistance in these other Enterobacteriaceae and speculated that *E coli* was a principal carrier of antimicrobial resistance genes in the fecal flora. Similarly, a separate study[15] suggested that *E coli* is a major reservoir of resistance traits in the coliform flora of cattle, and *E coli* has been shown to have the ability to horizontally transfer resistance genes to other genera.[16] For these reasons, *E coli* has been used as an indicator organism when monitoring antimicrobial resistance in gram-negative bacteria.[17,18] Specifically, therefore, the purpose of the study reported here was to compare antimicrobial susceptibility patterns of *E coli* isolates from dairy farms using organic versus conventional production methods.

Materials and Methods

Sample collection—Fecal samples were collected from dairy cattle on 30 dairy farms using organic production methods and 30 dairy farms using conventional production methods in southwestern Wisconsin. The organic dairy farms were members of an organic dairy association that consisted of 325 dairy farms. All organic farms had been certified by a USDA-accredited certification agency as not having used antimicrobials on cows for at least 3 years prior to the start of

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the study. For each organic farm included in the study, the geographically closest conventional dairy farm whose owner agreed to participate was included in the study.

The study was conducted during 2000 and 2001. All participating dairy farms were visited twice during the study, once in March and once in September. During each visit, fecal samples were collected from 5 lactating cows and 5 calves that were less than approximately 6 months old. Animals that had diarrhea or were being treated for another illness were excluded. For the lactating cows, fecal samples were obtained by walking among the cows and waiting for them to defecate. Each fecal sample was taken from a freshly voided fecal pile, taking care to not contact the ground beneath the sample. For the calves, fecal samples were obtained directly, following anal stimulation.

To avoid cross-contamination, a sterile latex glove was used to collect each fecal specimen. Approximately 5 g of each fecal sample was placed in a tube containing Cary-Blair transport medium,[a] and another 5 g was placed in a sterile plastic tube. Specimens were placed on ice and sent to the Michigan Department of Community Health Laboratory by overnight courier service.

During the first visit to each dairy farm, information on management practices (eg, milk production, milking practices, housing, incidence of major dairy diseases, and medical treatments administered) was obtained by use of an orally administered questionnaire.

Bacterial culture methods—Fecal samples were processed within 72 hours after collection by streaking directly on MacConkey agar plates.[b] Plates were incubated at 35°C and examined after 24 hours. If no suspect *E coli* colonies were evident, a portion of the original fecal sample was inoculated in a MacConkey broth tube and the tube was incubated at 35°C for 24 hours and then streaked on a MacConkey agar plate. Representative lactose-fermenting colonies were selected and subcultured. Isolates were confirmed to be *E coli* by means of standard biochemical testing (ie, acid and gas production when grown on triple sugar iron agar; positive reactions for methyl red and indole; and negative reactions for Voges-Proskauer, Simmons citrate, hydrogen sulfide, urease, and phenylalanine).[19,20]

Antimicrobial susceptibility testing—For each *E coli* isolate, **minimum inhibitory concentrations (MICs)** of 17 antimicrobial agents were determined as recommended by the NCCLS.[21] A commercially available semiautomatic microbroth dilution test[c] was used in accordance with the manufacturer's instructions. Commercially available strains of *E coli* (ATCC 25,922), *Staphylococcus aureus* (ATCC 29,213), and *Pseudomonas aeruginosa* (ATCC 27,853) were used for quality control.

Data analysis—Clinical MIC breakpoints recommended by the NCCLS were used to classify isolates as susceptible or resistant to each of the 17 antimicrobials. For this analysis, isolates with intermediate susceptibility were categorized as being susceptible. Logistic regression was then used to estimate the odds ratio for resistance to each antimicrobial, comparing isolates from conventional dairy farms with isolates from organic farms. Animal age (cow vs calf) and season (September vs March) were included in these analyses as possible confounders. Logistic regression analysis was also used to determine whether various management factors (ie, number of cattle, amount of milk produced, whether grazing was intensive, whether animals had been purchased during the past year, mastitis rate, calf mortality rate, and number of years the farm had been using organic production methods) were predictive of the prevalence of antimicrobial resistance. The Mantel-Haenszel mean score statistic[22] was used to compare numbers of *E coli* isolates resistant to 0, 1, 2, and ≥ 3 antimicrobials between conventional and organic dairy farms. All analyses were performed with standard software.[d] Values of $P < 0.05$ were considered significant.

Results

A total of 1,191 fecal samples were collected from the dairy farms (5 farms had < 5 calves from which samples could be collected at the time of farm visits). *Escherichia coli* was isolated from 1,121 of the 1,191 (94%) fecal samples. *Escherichia coli* was not isolated from 45 of the 596 (7.6%) fecal samples from organic dairy farms and was not isolated from 25 of the 595 (4.2%) fecal samples from conventional dairy farms.

All 30 organic dairy farms had converted to organic production methods > 3 years prior to the study (mean, 8 years; range, 3 to 15 years). Owners of all 30 organic farms indicated that no antimicrobials had been administered to dairy cows since they had adopted organic production methods. However, owners of 4 organic farms reported that antimicrobials were administered to calves with severe diarrhea or pneumonia.

Owners of 26 of the 30 conventional dairy farms reported that cows routinely received antimicrobial infusions into the udder at the cessation of each lactation cycle. Cephapirin and penicillin were the most frequently used products. Owners of 18 conventional dairy farms reported using udder infusions of antimicrobials for the treatment of clinical mastitis. In addition, owners of 8 conventional farms indicated that systemically administered antimicrobials (eg, penicillin and oxytetracycline) were used to treat cows with severe clinical mastitis.

None of the *E coli* isolates were resistant to ceftriaxone, amikacin, nalidixic acid, or ciprofloxacin with the NCCLS breakpoints **(Table 1)**. However, 5 isolates from calves on 3 conventional dairy farms had reduced susceptibility to ceftriaxone (MIC ≥ 4 µg/mL). These 5 isolates also had reduced susceptibility to ceftiofur (MIC ≥ 4 µg/mL) and were resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin, cefoxitin, streptomycin, chloramphenicol, tetracycline, and sulfamethoxazole.

Twenty-seven isolates resistant to chloramphenicol were isolated from calves on 18 farms. All but 5 of these isolates were from calves on conventional dairy farms, and all 27 isolates were resistant to multiple antimicrobials (median, 5 antimicrobials; range, 3 to 11 antimicrobials). Of these 27 chloramphenicol-resistant *E coli* isolates, 26, 25, 22, and 22 were resistant to sulfamethoxazole, tetracycline, streptomycin, and kanamycin, respectively.

In logistic regression analyses, farm type (organic vs conventional) and animal age (cow vs calf) were significantly associated with odds that *E coli* isolates would be resistant to various antimicrobials. Season (September vs March) and the interaction between farm type and animal age were not included in the final model because they were not significant. Number of cattle, amount of milk produced, whether grazing was intensive, whether animals had been purchased during

Table 1—Results of antimicrobial susceptibility testing of 1,121 *Escherichia coli* isolates obtained from fecal samples from cows and calves on dairy farms in Wisconsin that used conventional or organic production methods.

		Conventional farms		Organic farms	
Antimicrobial	**MIC (µg/mL)**	**No. of cow isolates**	**No. of calf isolates**	**No. of cow isolates**	**No. of calf isolates**
Ampicillin	≤ 2	109	74	105	102
(breakpoint MIC, 32 µg/mL)	4	158	112	147	121
	8	10	11	12	7
	16	2	1	2	3
	32	0	2	0	1
	> 32	7	84	4	47
Amoxicillin-clavulanic acid	≤ 0.5	0	0	1	0
(breakpoint MIC, 32 µg/mL)	1	11	2	9	11
	2	49	27	44	37
	4	174	144	176	155
	8	45	96	34	70
	16	6	6	5	4
	32	1	2	1	3
	> 32	0	7	0	1
Cephalothin	2	9	2	7	11
(breakpoint MIC, 32 µg/mL)	4	60	53	43	51
	8	154	153	147	161
	16	54	62	64	44
	32	4	5	4	8
	> 32	5	9	5	6
Cefoxitin	≤ 4	247	236	216	252
(breakpoint MIC, 32 µg/mL)	8	36	36	49	23
	16	2	5	5	4
	32	1	3	0	2
	> 32	0	4	0	0
Ceftiofur	0.5	283	276	266	277
(breakpoint MIC, 8 µg/mL)	1	0	1	0	3
	2	1	1	0	1
	4	1	1	3	0
	8	0	4	1	0
	16	0	1	0	0
	> 16	1	0	0	0
Ceftriaxone	≤ 0.25	284	278	270	280
(breakpoint MIC, 32 µg/mL)	0.50	1	1	0	1
	2.00	1	0	0	0
	4.00	0	2	0	0
	8.00	0	3	0	0
Streptomycin	≤ 32	279	186	263	220
(breakpoint MIC, 64 µg/mL)	64	5	46	4	35
	128	1	27	1	18
	256	1	22	2	7
	> 256	0	3	0	1
Kanamycin	≤ 16	281	179	269	230
(breakpoint MIC, 64 µg/mL)	32	0	1	0	0
	64	4	104	1	51
Gentamicin	≤ 0.25	25	16	21	21
(breakpoint MIC, 16 µg/mL)	0.5	198	185	192	192
	1	59	57	54	59
	2	2	3	2	2
	4	0	1	1	0
	8	0	2	0	0
	16	2	5	0	0
	> 16	0	15	0	7
Apramycin	≤ 2	12	19	15	10
(breakpoint MIC, 32 µg/mL)	4	183	184	171	179
	8	81	72	76	87
	16	7	5	5	5
	32	2	1	2	0
	< 32	1	3	1	0
Amikacin	≤ 4	282	282	269	277
(breakpoint MIC, 64 µg/mL)	8	4	2	1	4
Chloramphenicol	≤ 4	261	252	249	267
(breakpoint MIC, 32 µg/mL)	8	25	10	21	9
	32	0	6	0	0
	> 32	0	16	0	5
Tetracycline	≤ 4	252	113	249	169
(breakpoint MIC, 16 µg/mL)	8	9	16	5	12
	16	0	2	1	4
	32	0	24	3	21
	> 32	25	129	12	75
Sulfamethoxazole	≤ 128	277	201	259	230
(breakpoint MIC, 512 µg/mL)	256	0	2	0	0
	512	0	15	4	7
	< 512	9	66	7	44
Trimethoprim-sulfamethoxazole	≥ 0.12	264	196	250	227
(breakpoint MIC, 4 µg/mL)	0.25	19	40	12	26
	0.5	2	22	6	21
	1	0	8	0	1
	2	0	1	0	0
	> 4	1	17	2	6
Nalidixic acid	≤ 4	264	266	253	269
(breakpoint MIC, 32 µg/mL)	8	22	18	17	12
Ciprofloxacin	≤ 0.015	283	278	269	280
(breakpoint MIC, 4 µg/mL)	0.03	2	6	1	1
	0.06	1	0	0	0

MIC = Minimum inhibitory concentration.
Isolates with an MIC greater than or equal to the breakpoint MIC were classified as resistant.

Table 2—Logistic regression analysis of the odds of resistance to various antimicrobials among *E coli* isolates obtained from cows and calves on dairy farms in Wisconsin that used conventional or organic production methods.

	Conventional farms	Organic farms		
Antimicrobial	No. resistant/No. susceptible	No. resistant/No. susceptible	Odds ratio	*P* value
Ampicillin	93/477	52/499	2.055	< 0.001
Amoxicillin-clavulanic acid	10/560	5/546	1.980	0.214
Cephalothin	23/547	23/528	0.969	0.918
Cefoxitin	8/562	2/549	3.997	0.081
Ceftiofur	6/564	1/550	5.910	0.101
Ceftriaxone	0/570	0/551	NA	NA
Streptomycin	105/465	68/483	1.755	0.002
Kanamycin	107/462	52/499	2.588	< 0.001
Gentamicin	22/548	7/544	3.241	0.008
Apramycin	7/563	3/548	2.262	0.239
Amikacin	0/570	0/551	NA	NA
Chloramphenicol	22/548	5/546	4.384	0.003
Tetracycline	180/390	116/434	2.009	< 0.001
Sulfamethoxazole	90/480	63/488	1.531	0.021
Trimethoprim-sulfamethoxazole	18/552	37/498	2.272	0.057
Nalidixic acid	0/570	0/551	NA	NA
Ciprofloxacin	0/570	0/551	NA	NA

The odds ratio represents the odds that an isolate from a conventional dairy farm would be resistant, compared with an isolate from an organic dairy farm. NA = Not applicable (no isolates were resistant).

Table 3—Frequency distribution of resistance to multiple antimicrobials among 1,120 *E coli* isolates from cows and calves on dairy farms in Wisconsin that used conventional or organic production methods.

	No. of cow isolates		No. of calf isolates	
No. of antimicrobials to which isolates were resistant	Conventional farms	Organic farms	Conventional farms	Organic farms
0	245	241	121	167
1	19	12	20	27
2	11	10	24	20
3	7	6	36	24
4	2	0	43	19
5	0	1	11	17
6	0	0	14	3
7	0	0	7	2
8	0	0	4	1
9	0	0	1	0
10	0	0	1	1
11	0	0	2	0
12	0	0	1	0
Total	**284**	**270**	**285**	**281**

For *E coli* isolates from cows, the frequency distribution of antimicrobial resistance was not significantly ($P = 0.434$) different between conventional and organic farms. For *E coli* isolates from calves, the frequency distribution of antimicrobial resistance was significantly ($P < 0.001$) different between conventional and organic farms.

the past year, mastitis rate, calf mortality rate, and number of years each farm had been using organic production methods were not significant predictors of the prevalence of antimicrobial resistance. Results of logistic regression analyses indicated that conventional dairy farms had significantly higher rates of resistance to ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, tetracycline, and sulfamethoxazole among *E coli* isolates than did organic dairy farms (**Table 2**).

Prevalence of resistance to multiple antimicrobials was not significantly different between *E coli* isolates from adult cows on conventional dairy farms versus adult cows on organic dairy farms (**Table 3**). However, prevalence of resistance to multiple antimicrobials was significantly ($P < 0.001$) higher among *E coli* isolates from calves on conventional dairy farms, compared with calves on organic dairy farms.

Discussion

In the present study, all fecal samples were collected from presumably healthy cows and calves that were not, to our knowledge, receiving treatment for any disease. This sampling protocol may have resulted in lower prevalences of antimicrobial resistance in the present study, compared with prevalence in a study[23] of samples submitted to a diagnostic laboratory, many of which likely came from animals that had recently been treated with antimicrobials. Similarly, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program reported a higher prevalence of resistant bacteria in samples submitted to a diagnostic laboratory, compared with samples collected from animals at slaughter.[17] For example, the Danish program found that 80% to 86% of *E coli* isolates from samples submitted to a diagnostic laboratory were resistant to ampicillin, but only 0% to 8% of *E coli* isolates from presumably healthy cattle at slaughter were resistant to ampicillin.[17]

As was the case in previous studies,[17,18] we avoided the difficult issue of clustering of isolates in cows within herds by examining only a single representative *E coli* isolate from each animal. A nondifferential selection bias may have existed if the *E coli* isolates that we were able to culture were, in some unknown way, not representative of all *E coli* isolates present in the fecal samples. However, any such nondifferential bias should not have affected our comparisons between organic and conventional dairy farms.

Assessing the level of antimicrobial use on conventional dairy farms participating in the present study was difficult. Despite our requests to do so, owners of conventional dairy farms usually did not record the details of antimicrobials used. Also, some owners did not know what treatments were rendered by their veterinarians. On the other hand, our assumption that cows in organic dairy herds were not given any antimicrobials was dependent on the owners' compliance with organic production rules. If there had been violations of the organic production rules, the dairy farm would have lost its organic status and would not have been able to sell its milk at the premium organic price.[24] Compliance with organic production rules was assumed to be high, in that conversion to organic dairy production may take approximately 2 years and organically produced milk sold for almost twice as much as conventionally produced milk during the time of the study.

The use of chloramphenicol in food-producing animals has been prohibited in the United States since January 1986, and this ban has been rigorously enforced by the FDA.[25] A previous study[26] of 48 *E coli* isolates from calves with diarrhea found that most of the resistant *E coli* isolates had enhancing efflux genes (*flo* and *cmlA*), which may have been disseminated via plasmids or mobile transposons. The authors suggested that extralabel use of florfenicol in the calves could have selected for these resistance traits. Florfenicol is structurally similar to chloramphenicol and was approved for treatment of bovine respiratory tract disease in 1996.[27] Resistance to chloramphenicol could also have been a result of coselection by other antimicrobials. Our finding of a high number of isolates resistant to multiple antimicrobials supports the possibility of coselection by antimicrobials other than chloramphenicol. Further research is required to identify on-farm selective pressures that have caused the chloramphenicol resistance trait to be preserved for so many years on both organic and conventional dairy farms.

For both organic and conventional dairy farms in the present study, the overall prevalence of antimicrobial resistance was higher among *E coli* isolates from calves than among isolates from adult cows. Calf milk replacers, which may contain antimicrobials such as chlortetracycline, oxytetracycline, and neomycin, are widely used by dairy producers in the United States.[13,28] However, although organic dairy producers may use antimicrobials in calves < 1 year old for treatment of life-threatening situations, they are prohibited from using medicated milk replacers. Thus, use of medicated milk replacers may possibly help explain the high prevalence of resistant *E coli* among calves on conventional dairy farms, but it does not explain the difference between cows and calves that we observed on the organic farms. Organic dairy producers usually treated sick calves with orally administered electrolyte solutions, whey products, and probiotic products, and only 4 of the 30 organic producers indicated that they had used antimicrobials to treat sick calves in the preceding year. Therefore, it appears that antimicrobials were rarely used on the organic dairy farms. Authors of a previous study[29] reported a high prevalence of resistant *E coli* in calves that did not receive any orally administered antimicrobials and found a continual turnover of *E coli* strains in the fecal flora, most of which were isolated only a few times. A higher prevalence of resistant *E coli* in calves, compared with cows, has also been found in organic and conventional dairy herds in Denmark, where the use of medicated milk replacer has been prohibited since the early 1970s.[e] A higher percentage of resistant bacteria has also been observed in young pigs, compared with adults.[30-32] Thus, animal age could be an important factor when investigating antimicrobial resistance in enteric *E coli* strains.

In our analyses, we did not find any significant association between management factors and prevalence of antimicrobial resistance in *E coli* isolates when controlling for farm type (organic vs conventional) and animal age. When we did not control for farm type and animal age in the logistic regression analysis, intensive grazing was a significant predictor of the prevalence of antimicrobial resistance. This most likely was because intensive grazing was more common among the organic dairy farms.

a. Medical Chemical Corp, Torrance, Calif.
b. Remel, Lenexa, Kan.
c. Sensititre, Trek Diagnostic Systems Inc, Cleveland, Ohio.
d. SAS, version 8.02, SAS Institute Inc, Cary, NC.
e. Bennedsgaard TW. *Antimicrobial resistance of Escherichia coli in conventional and organic dairy herds in Denmark*. PhD dissertation, Department of Animal Husbandry and Animal Health, Royal Veterinary and Agriculture University, Frederiksberg, Denmark, 2003.

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