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Sub-therapeutic Use of Antibiotics and Prevalence of Antibiotic Resistant Bacteria on Swine Farms

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Abstract: This study was undertaken to compare the prevalence of antibiotic resistant bacteria (ARB) in manure, soil and dog fecal samples collected from swine farms using antibiotics (AU) and not using antibiotics (NAU) sub-therapeutically. A total of 12 farms (6 of each type) were surveyed for this study. All samples were screened for the presence of ARB by plating on Mueller Hinton II agar with or without antibiotic (tetracycline, tylosin, or monensin). Results showed a significantly higher ($p<0.05$) prevalence of tetracycline resistant bacteria (TC^r) and monensin resistant bacteria (MON^r) in manure samples from AU farms than from NAU farms. However, there was no significant difference in the prevalence of tylosin resistant bacteria (TYL^r) in manure samples between AU and NAU farms. Also, the difference in the prevalence of TC^r, TYL^r and MON^r in either soil or dog fecal samples between the two types of farm was non-significant. Antimicrobial susceptibility profile of common Gram negative bacterial isolates from these samples showed only small differences between AU and NAU farms. In general, isolates from AU farms showed higher resistance to ampicillin, spectinomycin, tetracycline and sulphonamides. *E. coli* isolates from dog fecal sample had significantly higher resistance from the AU than the NAU farms thus raising some concerns on the potential of ARB spread to humans on the farm. We also conclude that higher prevalence of ARB in swine manure samples from AU farms is not spreading to the terrestrial environment following land application of manure.

Key words: Antibiotic resistant bacteria, swine farms, manure, soil, dog feces

INTRODUCTION

Increased resistance of pathogenic and non-pathogenic bacteria to different antibiotics has become an important issue. Antimicrobial resistance is not a new phenomenon since penicillin resistant *Escherichia coli* strains were reported as early as 1950, soon after the introduction of antibiotics. However, the rapid pace at which large numbers of bacteria are acquiring resistance to newer antibiotics has forced this issue to the forefront of public policy debate in recent years (Shea, 2003). Infections caused by antibiotic resistant bacteria (ARB) result in higher rates of hospitalization, higher health care costs and even death in some cases due to treatment failure (Linden, 1998).

One of the factors responsible for the development of antibiotic resistance in microbial population is the use of antibiotics in food animals for therapeutic and non-therapeutic purposes (Smith *et al.*,

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2002). Non-therapeutically, antibiotics are used to promote weight gain in animals as well as to improve general hygiene in the barn (Schwarz, 2001). As much as 90% of the antibiotics used in agriculture are used as prophylactic agents and growth promoters rather than to treat infections (OTA, 1995). The typical antibiotic dose varies from 1 to 200 g per ton of feed depending upon the type and age of animal and the type of antibiotic administered (Nwosu, 2001; Kumar *et al*., 2005). Most of the antibiotics are poorly absorbed in the gastrointestinal tract of animals and 25 to 75% of the orally administered antibiotics are excreted unaltered in the feces (Feinman and Matheson, 1978).

Non-therapeutic use of antibiotics as feed additives can lead to increased antimicrobial resistance in microflora associated with animals and ultimately to zoonotic pathogens (van den Bogaard *et al*., 2000; Wegener, 2003). Studies have indicated that antimicrobial use in food animal production systems promotes the development and subsequent dissemination of resistant organisms to humans (National Research Council, 1999). The transmission on of ARB between animals and humans is not limited to agents of zoonotic diseases; the selection of resistant population of opportunistic human pathogens and the possible transmission of resistant determinants to human pathogens could have undesirable consequences on human health (Boerlin *et al*., 2001). van den Bogaard and Stobberingh (2000) reported high resistance to a number of antibiotics in *Escherichia coli* isolated from slaughterhouses and concluded that the use of a particular antibiotic caused higher prevalence of resistance to that antibiotic. Increased levels of ARB in animals can also lead the farmers to acquire them from their animals (Aubry-Damon *et al*., 2004; Marshall *et al*., 1990). Concerned over the negative impact of sub-therapeutic use of antibiotics, European Union countries have decided to stop the subtherapeutic feeding of antibiotics completely by the end of 2006 (Bywater, 2005).

Macrolide (tylosin) and tetracycline (chlortetracycline and oxytetracycline) are widely used for growth promotion in pigs (Jackson *et al*., 2004; Mathew *et al*., 2001) and the most common route of antibiotic administration in pigs is through the feed (National Animal Health Monitoring System, www.aphis.usda.gov/vs/ceah/cahm). However, little information is available on the impact of this practice on the emergence and spread of ARB in the environment. The goal of this study was to compare the prevalence of ARB on two types of swine farms; those that use antibiotics as feed supplements and those where no antibiotics are used as feed supplement. The specific objective was to characterize and compare the prevalence of ARB in manure, agricultural soil where manure has been regularly applied and dog fecal samples on pig farms that use or do not use antibiotics sub-therapeutically. Agricultural soils are included in the study to determine whether or not ARB is spreading to the terrestrial environment as a result of manure application. The dog fecal samples were tested to determine whether or not ARB is spreading to pets on the farm and perhaps indirectly to humans that are coming in contact with these pets.

MATERIALS AND METHODS

Sample Collection and Screening

All the swine farms selected in the present study were located in Minnesota. Samples of manure, soil and dog feces were collected from six swine farms that were using antibiotics as feed additives (AU farms) and six other farms where no antibiotics have been used as feed additive for at least three years (NAU farms). The samples were collected in sterile polypropylene containers, transported to the laboratory on ice and were tested within 24 h of collection. The age of the manure could not be accurately ascertained but probably varied from one day (samples from animal barns) to nine months (samples from manure lagoon/pit). Soil samples were collected at a depth of 3-6 inches from fields where manure had been regularly applied for the last three or more years. Fresh dog feces (less than 24 h old) were collected from all farms. Only one fecal sample was collected from each farm and none of the dog sampled was under medication (antibiotic treatment) at the time of sample collection.

All producers were interviewed regarding their manure management practices on the farm and the use of antibiotics. At the time of sampling, none of the 12 swine farms were using antibiotics for therapeutic purposes. Also, tylosin and chlortetracycline were the two most common antibiotics used on all AU swine farms. Other antibiotics used were bacitracin, lincomycin and sulfa drugs.

To quantify the prevalence of ARB, a 10% suspension of each sample was prepared by suspending 1 g of sample in 9 mL of buffered peptone water (BPW, pH 7.0) followed by vortexing to make a homogenous suspension. Serial 10-fold dilutions of this suspension were prepared in BPW and 50 μL of 10^{-3} to 10^{-8} dilutions were plated on Muller Hinton II agar plates (MHA; Becton Dickinson) containing tetracycline (20 μg mL^{-1}), tylosin (10 μg mL^{-1}), or monensin (6 μg mL^{-1}). As a control, all dilutions were plated simultaneously on MHA plates without antibiotics. The plates were then incubated at 37°C for 24 h and the number of colony forming units (CFU) on each plate was counted manually. The percent of ARB in each sample was calculated as the ratio of CFU growing on antibiotic plate to the number of CFU on antibiotic-free control plate x100 (van den Bogaard *et al*., 2000).

Antibiotic concentrations used for the screening of ARB were selected on the basis of established minimum inhibitory concentration (MIC) values as per NCCLS guidelines (NCCLS, 2002). Since there are no standard MIC values for monensin, the concentration used was based on the published literature (van den Bogaard and Stobberingh, 2000).

Bacterial Identification and Minimum Inhibitory Concentrations (MIC)

Phenotypically different colonies of ARB from each antibiotic plate (4-5 colonies per plate) were picked, purified and stored on tryptic soy agar slants (TSA; Becton Dickinson). Isolated bacteria were identified using API 20E and API 20NE identification strips (BioMereiux, France) and their antimicrobial susceptibility was determined to 14 different antibiotics belonging to nine different groups e.g., macrolide (erythromycin and tilmicosin), tetracycline (chlortetracycline and oxytetracycline), β-lactam (penicillin and ampicillin), chloramphenicol (florfenicol), aminoglycoside (neomycin and gentamicin), fluoroquinolone (tiamulin), lincosamide (clindamycin), sulphonamide (sulphadimethoxine and trimethoprim:sulfathiazole) and aminocyclitols (spectinomycin). Antimicrobial susceptibility was determined by the broth dilution method (NCCLS, 2002) using Sensititer CMV1 ABPF antibiotic sensitivity plates (TREK Diagnostics, Cleveland, OH). The procedure involved inoculating the Sensititer plates with 0.5 McFarland adjusted inoculum as per the product insert and incubating it at 37°C for 24 h. Minimum antibiotic concentration that completely inhibited bacterial growth (MIC) was determined using a Sensititer plate reader (Sensitouch, TREK Diagnostics, Cleveland, OH).

Statistical Analysis

Data on the prevalence of ARB were analyzed using the analysis of variance (ANOVA) with Statistical Analysis System software (SAS Institute, 1996). ARB data were analyzed separately for each of the three antibiotics. The experimental design for ANOVA was a split-plot design with antibiotic use as the main effect and matrix (manure, soil and dog feces) as the secondary effect. Significance of differences among treatments was analyzed at $p<0.05$. To achieve normality, data were transformed by taking the arcsin of the square root of percent antibiotic resistance. Transformed data were analyzed by ANOVA and the mean values reported are the values obtained after reverse transformations of the mean values obtained from ANOVA. Differences in antibiotic resistance profile of selected bacterial isolates from AU and NAU farms were tested using the Student's t test (SPSS, 1996). Because of the small number of antibiotics tested for each antibiotic group, differences in antibiotic resistance profile were evaluated on aggregated values over all 14 antibiotics. Description of the differences in antibiotic resistance profile between the AU and the NAU for each antibiotic or a group of antibiotics is therefore qualitative.

RESULTS

Prevalence of ARB

Averaged over all three matrices, the prevalence of tetracycline resistant bacteria (TCr) was significantly higher on the AU than the NAU farms ($p<0.05$, Table 1). However, there was an interaction between the antibiotic usage and the matrix for TC^r. Higher TC^r resistance on the AU farms was mainly due to higher TC^r in AU manure samples (Table 1). Prevalence of TC^r in soil and dog fecal samples between the two types of farm was not significantly different ($p<0.05$). There was no difference in the prevalence of tylosin resistant bacteria (TYL^r) between the two types of farms. Irrespective of the farm type, prevalence of TYLr was significantly higher ($p<0.05$) in manure (30.2%) samples compared to soil (4.7%) and dog fecal (9.2%) samples. However, the difference in the prevalence of TYL^r between the soil and the dog feces was not significant.

Irrespective of the matrix, monensin resistant bacteria (MON^r) were more prevalent ($p<0.05$) on the AU than the NAU farms. Irrespective of the farm type, there was no difference in MON^r between the soil and dog fecal samples.

Bacterial Identification

A total of 137 and 113 ARB colonies were picked from the AU and NAU samples, respectively (Table 2). Of these, 57 (AU farms) and 53 (NAU farms) isolates could be identified using the API identification system. The distribution of identified bacterial isolates for the AU farms was: 31 of 72 in manure, 9 of 29 in soil and 17 of 36 in dog fecal samples. Similarly for the NAU farms, the distribution of identified isolates was: 24 of 59 in manure, 11 of 26 in soil and 18 of 28 in dog fecal samples (Table 2). Irrespective of antibiotic usage on the farm, *Pasteurella* sp. and *E. coli* were the predominant isolates from manure and dog feces, respectively. The predominant isolate from the AU soils was *Pseudomonas* sp. while from the NAU soils it was *Serratia* sp.

Resistance Profile of Bacterial Isolates

Manure

Pasteurella sp. from the AU farm (n = 11) showed high resistance to sulphonamide (except trimethoprim:sulfa) and tetracycline groups of antibiotics whereas those from the NAU farms (n = 7) showed higher resistance to macrolide and lincosamide groups of antibiotics (Table 3). All *Pasteurella* isolates from the NAU farms were susceptible to β-lactam antibiotics whereas 3 of 11 isolates from the AU farms were resistant to this group of antibiotics. Except for one isolate from the NAU farm, which showed resistance to gentamicin, all *Pasteurella* isolates from the AU and NAU farms were susceptible to aminoglycosides (neomycin and gentamicin).

Table 1: Analysis of variance (pr>F) on the prevalence of Antibiotic Resistant Bacteria (ARB) for three antibiotics

	Mean		
Variable	Tetracycline	Tylosin	Monensin
Antibiotic user (AU)			
Manure (M)	37.00a[a]	37.96a	32.18a
Soil (S)	0.23bc	9.01bc	19.62ac
Dog feces (D)	3.88b	8.23bc	16.84ac
Non-Antibiotic user (NAU)			
Manure	4.69b	22.93ac	7.61ac
Soil	0.00c	1.74b	2.44bc
Dog feces	3.80b	10.20bc	18.41ac
Statistics			
Usage (AU×NAU)	0.0045*	0.2019	0.0071*
Matrix (M×S×D)	<0.001*	0.0026*	0.6183
Usage×matrix	0.0040*	0.473	0.4578

*Statistically different at $p<0.05$, [a]For a given antibiotic, means followed by a different letter are significantly different ($p<0.05$)

Table 2: Identity of antibiotic resistant bacteria isolated from antibiotic user (AU) and non-antibiotic user (NAU) swine farms (n = number of isolates)

Antibiotic user farm (n = 137)	Non-antibiotic user farm (n =113)
Manure (31/72)[a]	**Manure (24/59)**
Pasteurella sp. (n = 11)	*Pasteurella* sp. (n = 7)
E. coli sp. (n = 6)	*Moraxella* sp. (n = 6)
Acinetobacter sp. (n = 4)	*Serratia* sp. (n = 5)
Moraxella sp. (n = 3)	*Alcaligenes* sp. (n = 2)
Yersinia sp. (n = 2)	*Yersinia* sp. (n = 1)
Pseudomonas sp. (n = 2)	*Acinetobacter* sp. (n = 1)
Brevibacterium sp. (n = 2)	*Brevibacterium* sp. (n = 1)
Pontoea sp. (n = 1)	*Stenotrophomonas* sp. (n = 1)
Soil (9/29)	**Soil (11/26)**
Pseudomonas sp. (n = 5)	*Serratia* sp. (n = 5)
Pasteurella sp. (n = 3)	*Pseudomonas* sp. (n = 3)
Moraxella sp. (n = 1)	*Pasteurella* sp. (n = 1)
Dog Feces (17/36)	*Acinetobacter* sp. (n = 1)
E. coli (n = 14)	*Stenotrophomonas* sp. (n = 1)
Pasteurella sp. (n = 1)	**Dog Feces (18/28)**
Klebsiella sp. (n = 1)	*E. coli* (n = 14)
Proteus sp. (n = 1)	*Acinetobacter* sp. (n = 1)
	Cedecea sp. (n = 1)
	Pontoea sp. (n = 1)
	Burkholderia sp. (n = 1)

[a]No. of isolates identified/ total number of isolates

All *Pasteurella* sp. isolates from both types of farms were resistant to up to 8 antibiotics (Table 4). Relatively, a small number of *Acinetobacter* sp. was isolated from both types of farms. This microorganism generally had higher resistance to sulphonamide and tetracycline groups of antibiotics irrespective of the farm type (Table 3). However, isolates from the AU farms had relatively higher resistance to clindamycin and spectinomycin. Two (50%) *Acinetobacter* sp. isolates from AU farm were resistant to up to 4 antibiotics while the other 2 were resistant to 8 antibiotics. The only isolate from NAU farm was resistant to 4 antibiotics (Table 4).

Most of the *Moraxella* sp. isolates from both types of farms showed resistance to macrolide, tetracycline and sulphonamide groups of antibiotics (Table 3). None of the isolates was resistant to ampicillin. Resistance was widespread among *Moraxella* sp. isolates from NAU farms and some of the isolates were resistant to as high as 13 antibiotics (Table 4). Averaged over 14 antibiotics, there was no difference in the percent resistance of *Pasteurella* sp. and *Acinetobacter* sp. between the AU and the NAU farms (Table 4) but the resistance was significantly higher ($p<0.05$) for *Moraxella* sp. from the NAU than the AU farms (Table 3).

Soil

Pseudomonas and *Pasteurella* isolates were the two most common isolates in soil samples from the AU and the NAU farms (Table 2). Resistance to sulphonamides was higher in *Pasteurella* isolates from the AU farms compared to those from the NAU farms (Table 3). The lone *Pasteurella* isolate from the NAU farm was susceptible to β-lactam, aminoglycoside and sulphonamide groups of antibiotics but was resistant to both macrolide. In addition, it was susceptible to chlortetracycline, florfenicol and spectinomycin but resistant to oxytetracycline, clindamycin and tiamulin. Only two of the three isolates of *Pasteurella* from the AU farms showed resistance to erythromycin and tilmicosin. All *Pseudomonas* isolates from both types of farms were found to be resistant to florfenicol and clindamycin. Most of the *Pseudomonas* isolates were susceptible to tetracycline and aminoglycoside groups of antibiotics, however one *Pseudomonas* sp. isolate (20%) from the AU farm was resistant to chlortetracycline and gentamicin. Also, all three isolates (100%) from the NAU farm were resistant to β-lactam while 80% of the isolates from the AU farm showed resistance to this group

Table 3: Percent resistance of the most common bacterial isolates to various antibiotics

		Microorganism					
		Pasteurella sp. (Manure)		*Acinetobacter* sp. (Manure)		*Moraxella* sp. (Manure)	
Antibiotics	Breakpoint[a] (μg mL^{-1})	AU[b] (n = 11)	NAU[c] (n = 7)	AU (n = 4)	NAU(n = 1)	AU (n =3)	NAU (n = 6)
Macrolide							
Erythromycin	≥8	18.2	57.1	0.0	0.0	100.0	100.0
Tilmicosin	≥32	9.1	57.1	0.0	0.0	66.7	100.0
Tetracycline							
Chlortetracycline	≥16	36.7	0.0	25.0	100.0	33.3	83.3
Oxytetracycline	≥16	81.8	42.8	75.0	100.0	66.7	83.3
β-Lactam							
Penicillin	≥16	18.2	0.0	0.0	0.0	33.3	50.0
Ampicillin	≥32	9.1	0.0	0.0	0.0	0.0	0.0
Phenicol							
Florfenicol	≥8	0.0	14.3	0.0	0.0	33.3	50.0
Aminoglycoside							
Neomycin	≥16	0.0	0.0	0.0	0.0	33.3	66.7
Gentamicin	≥16	0.0	14.3	0.0	0.0	33.3	50.0
Fluoroquinolone							
Tiamulin	≥32	27.3	28.6	75.0	0.0	33.3	83.3
Lincosamide							
Clindamycin	≥4	54.5	85.7	75.0	0.0	66.7	100.0
Sulphonamide							
Sulphadimethoxine	≥512	100.0	71.4	100.0	100.0	100.0	100.0
Trimethoprim: sulpha	≥2:38	27.3	28.6	25.0	0.0	100.0	50.0
Aminocyclitols							
Spectinomycin	≥128	18.2	42.5	50.0	0.0	66.7	50.0
Mean Resistance		28.6	31.6	30.4	21.4	54.8	69.0*

		Microorganism					
		Pasteurella sp. (Soil)		*Pasteurella* sp. (Soil)		*E. coli* (Dog feces)	
Antibiotics	Breakpoint[a] (μg mL^{-1})	AU (n = 3)	NAU (n = 1)	AU (n = 5)	NAU (n = 3)	AU (n = 14)	NAU (n = 14)
Macrolide							
Erythromycin	≥8	66.7	100.0	100.0	100.0	100.0	100.0
Tilmicosin	≥32	66.7	100.0	100.0	100.0	100.0	100.0
Tetracycline							
Chlortetracycline	≥16	0.0	0.0	20.0	0.0	71.4	35.7
Oxytetracycline	≥16	33.3	100.0	0.0	0.0	78.6	35.7
β-Lactam							
Penicillin	≥16	33.3	0.0	80.0	100.0	100.0	100.0
Ampicillin	≥32	33.3	0.0	80.0	100.0	64.3	21.4
Phenicol							
Florfenicol	≥8	33.3	0.0	100.0	100.0	0.0	14.3
Aminoglycoside							
Neomycin	≥16	33.3	0.0	0.0	0.0	7.14	7.14
Gentamicin	≥16	0.0	0.0	20.0	0.0	0.0	7.14
Fluoroquinolone							
Tiamulin	≥32	33.3	100.0	100.0	100.0	100.0	100.0
Lincosamide							
Clindamycin	≥4	66.7	100.0	100.0	100.0	100.0	100.0
Sulphonamide							
Sulphadimethoxine	≥512	100.0	0.0	100.0	100.0	71.4	64.3
Trimethoprim: sulpha	≥2:38	100.0	0.0	80.0	33.3	7.1	0.0
Aminocyclitols							
Spectinomycin	≥128	33.3	0.0	40.0	0.0	42.8	14.3
Mean resistance		45.2	35.7	65.7	59.5	60.2*	50.0

[a]Minimum inhibitory concentration of antibiotic (NCCLS, 2002), [b] AU = Antibiotic user farm (total number of isolates), [c] NAU = Non-antibiotic user farm (total number of isolates) * Statistically significant at $p<0.05$

Table 4: Antibiotic resistance profile of common bacterial isolates

	Isolates resistant to number of antibiotics (%)											
	Manure						Soil				Dog Feces	
	Pasteurella sp.		*Acinetobacter* sp.		*Moraxella* sp.		*Pseudomonas* sp.		*Pasteurella* sp.		*E. coli*	
No. of antibiotics	AU[a] (n = 11)	NAU[b] (n = 7)	AU (n = 4)	NAU (n =1)	AU (n =3)	NAU (n =6)	AU (n =5)	NAU (n = 3)	AU (n = 3)	NAU (n = 1)	AU (n =14)	NAU (n = 14)
0-4	63.6	57.1	50.0	100.0	33.3	0.0	0.0	0.0	33.3	0.0	0.0	0.0
5-8	36.3	42.0	50.0	0.0	33.3	50.0	20.0	66.6	33.3	100.0	50.0	78.5
9-12	0.0	0.0	0.0	0.0	33.3	16.6	80.0	33.3	33.3	0.0	50.0	21.4
13-14	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0

[a] AU = Antibiotic user farm (total number of isolates), [b] NAU = Non-antibiotic user farm (total number of isolates)

of antibiotics. Resistance to sulphonamides was higher in *Pseudomonas* isolates from the AU farms as compared to those from the NAU farms. Averaged over all 14 antibiotics, there was no difference in the antibiotic resistance of *Pasteurella* and *Pseudomonas* isolates between the AU and the NAU farms (Table 4).

Dog Feces

E. coli was the dominant isolate identified in dog fecal samples from the AU and the NAU farms (Table 2). In general, *E. coli* isolates from the AU farms showed higher resistance to tetracycline and sulphonamide groups of antibiotics (Table 3). Resistance to macrolide and fluoroquinolone groups of antibiotics was comparable between isolates from the AU and the NAU farms. All *E. coli* isolates from the AU farm were susceptible to florfenicol but two isolates (14.3%) from the NAU farm were found to be resistant to these antibiotics (Table 3). Resistance to clindamycin was comparable (100%) in isolates from both types of farms but 42.8% of the isolates from the AU farm were resistant to spectinomycin as compared to 14.3% of the isolates from the NAU farm. Averaged over all 14 antibiotics, differences in the antibiotic resistance profile of *E. coli* isolated from the AU and the NAU farms were significant (Table 4). Most of the *E. coli* isolates (78.5%) from NAU farms were resistant to up to 8 antibiotics whereas 50% of the isolates from AU farms were found to be resistant to this many antibiotics (Table 4).

DISCUSSION

Widespread antimicrobial resistance in various pathogenic and non-pathogenic bacteria is believed to be due to the overuse of antibiotics in food-animal production. In this study, the prevalence of TC^r and MON^r was significantly higher ($p<0.05$) from the AU farms than the NAU farms. Higher TC^r in the AU farms was mainly due to higher TC^r in manure samples. According to the Union of Concerned Scientist (2001), tetracycline use in swine production over the last two decades has increased even though overall use of antibiotics in swine production has decreased by 11%. Thus, higher tetracycline resistance in manure samples from AU swine farms may be due to increased use of tetracycline in swine production. These results are consistent with findings of Langlois *et al.* (1983) who reported higher tetracycline resistance in fecal coliforms isolated from swine herds that were being fed antibiotics.

Present results on the lack of difference in the prevalence of TYLr in manure samples between AU and NAU farms are similar to the findings of Davies and Roberts (1999), who did not find any correlation between tylosin resistance in *Enterococcus faecium* and tylosin feeding. Also, Mathew *et al.* (2001) reported that exclusion of antibiotics in swine production decreases antimicrobial resistance in *E. coli* but does not eliminate it. Since most of the producers in the NAU group have shifted to 'no antibiotic use practice' only in the last three to four years, additional time may be needed

to purge the residual effects of antibiotic use. In a Danish study, decreased levels of antibiotic resistance were observed in fecal enterococci six years after the ban on the use of antimicrobials as growth promoters (Aarestrup *et al*., 2001). Marshall and Levy (2004) also noted that although resistance is acquired rapidly, its loss in the absence of a given antibiotic is a slow process. Although monensin is not used for growth promotion in pigs, the results of the present study showed higher prevalence of MON^r in AU samples than in NAU farms (Table 1). This could be because of cross resistance as a result of use of other antibiotics. Also, it is important to note that there are no standard MIC values for monensin and the antibiotic concentration used is based on published reports. It is possible that monensin concentration used were lower.

Large variation was observed in antibiotic resistance patterns of different bacterial isolates in manure samples from the AU and the NAU farms. This may be in part because of low numbers of isolates tested in this study. The low numbers of bacteria were mainly because we selected only Gram negative bacteria for susceptibility testing. For soil and dog fecal samples, no significant difference was observed in the prevalence of TC^r or TYL^r between the AU and the NAU farms. These results indicate that although the prevalence of TC^r was higher in swine manure from the AU farms, this resistance does not appear to spread to the terrestrial environment. Lack of differences in soil ARB could be because of the difficulty of culturing all soil bacteria and/or low survivability of manure bacteria in soil. In a Danish study, Sengelov *et al*. (2003) observed only temporary increase in tetracycline resistance as a result of pig manure application, indicating that tetracycline resistance levels in soil are temporarily influenced by the addition of swine manure.

Present results on the prevalence of TYL^r in soil samples from two types of farms are in 288 contrast to those of Onan and LaPara (2003) who reported high proportion of TYL^r (7.2-16.5%) in farms where sub-therapeutic antibiotic use was practiced as compared to farms where no antibiotics were used (0.7-2.5%). These differences could be due to mixed animal populations studied by Onan and LaPara (2003) while our study was limited only to pigs. Another possibility could be the differences in the methodology used for isolating and identifying ARB in the two studies.

Overall, bacterial isolates from AU farms showed higher resistance to ampicillin, spectinomycin, tetracycline and sulphonamides. These results are similar to those reported by Kim *et al*. (2005) who found higher resistance to tetracycline and apramycin in *E. coli* isolates from piglets fed these antibiotics as feed additives. Similarly, van den Bogaard and Stobberingh (2000) reported higher oxytetracycline and trimethoprim resistance in *E. coli* isolated from pigs that had been fed with these antibiotics. Present results on higher tetracycline resistance in *Pseudomonas* sp. from the AU farm are in agreement with the findings of Jensen *et al*. (2001) who observed significant differences in the prevalence of tetracycline resistant *Pseudomonas* sp. before and after the spread of animal manure. There was no difference in the prevalence of TC^r and TYL^r in dog fecal samples collected from either type of farm. However, tylosin resistance was widespread as compared to tetracycline resistance. This could be because most of the isolates from dog fecal samples were identified as *E. coli* and this organism is inherently resistance to tylosin. *E. coli* isolates from the AU farms showed higher resistance to four major groups of antibiotics namely: tetracycline (chlor- and oxytetracycline), β-lactam (ampicillin), sulphonamide (sulphadimethoxine and trimethoprim:sulfa) and aminocyclitols (spectinomycin). This suggests that although percent prevalence of ARB was the same in dog fecal samples from both types of farms, the resistance profile was influenced by the antibiotic used. Resistance to tiamulin was the same in isolates from both types of farms. In their study with laboratory animals, Hansen and Velschow (2000) found higher resistance to this antibiotic in all *E. coli* isolates although none of the animal was being fed with this antibiotic. Higher resistance in *E. coli* isolated from pet animals on farms using antibiotics is a matter of concern as these animals can act as a source of ARB to humans (Guardabassi *et al*., 2004).

CONCLUSIONS

The results indicate that tetracycline use as feed additive is leading to the emergence of ARB in manure samples. However, no significant difference was observed in the prevalence of ARB in soil and dog fecal samples suggesting that this resistance may not be spreading to the soil and to pets on the farm. Comparison of individual bacterial species in manure, soil and dog fecal samples between the AU and the NAU farms showed little significant differences, which could be due to large variability among a small number of isolates in each matrix. *E. coli* in dog fecal samples from the AU farm, however, had higher resistance than from the NAU farms thus raising some concern on its potential to spread to humans on the farm.

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