RESEARCHREPORT

PORK SAFETY

Title: Associations among therapeutic and in-feed antimicrobial use and resistance in fecal commensals and pathogens of swine.
NPB # 98-213

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I. Abstract:

Antimicrobials are used in pork production for treatment and control of infectious disease and in some cases for promotion of growth and disease prevention. While these drugs are valuable, there have been concerns expressed about their role in promoting resistance among enteric bacteria. *E. coli* are important enteric pathogens and commenals of animals and humans and are important indicators of antimicrobial resistance.

There were two main components to this study: the first was a statistical analysis of a large body of resistance data collected in 1992 from weaner pigs, and the second was a follow-up investigation of the same farms and an analysis of temporal changes in resistance that could be attributed to on-farm antibiotic use. In the first component, 4356 *Escherischia coli* isolates were tested for resistance to seven antimicrobial drugs (ampicillin, carbadox, gentamicin, nitrofurantoin, spectinomycin, sulfisoxazole, and tetracycline) using a hydrophobic-grid membrane filter method according to N.C.C.LS methods where applicable. The in-feed addition of a penicillin, carbadox, a sulphonamide and a tetracycline to weanling pig rations ("starter rations") was associated with increased risk of resistance to ampicillin, carbadox, sulfisoxazole and tetracycline, respectively. Individual weanling pig treatments were significant only in the ampicillin model. Management and housing factors were also significantly associated with the risk of resistance but without any consistent pattern. These results indicate that antimicrobial exposure to groups of pigs through feed is more consistently associated with increased risk of resistance among *E. coli* than individual animal treatment.

In the second component of the study, antimicrobial use data and an archive of fecal samples collected from 32 farms in 1992, along with a new series of comparable data from 1999, were used to identify temporal changes in the resistance pattern of *E. coli* and associations with antimicrobial use. Among the 32 farrow-to-finish farms sampled in 1992, 5 were no longer in business, and 10 changed their management practice to segregated early weaning or other system. No resistance was observed in any of the 900 *E coli* isolates from both years, to amikacin, ciprofloxacin, florfenicol and nalidixic acid. Compared with 1992, the proportion of *E.*

coli resistant to nitrofurantoin declined significantly ($p < 0.01$). Although the prevalence of resistance increased in 14 of the 23 pairs of prevalences analyzed, only in 4 final regression models was this increase statistically significant (cephalothin, chloramphenicol and tetracycline at 8 and 16 μg/ml). In-feed administration of an antimicrobial was significant in several logistic regression models of resistance (i.e. ampicillin, carbadox, kanamycin, nitrofurantoin, spectinomycin, sulfisoxazole and tetracycline). However, individual treatment factors and in-water administration of antimicrobials were significant only in streptomycin and kanamycin models, respectively.

These studies provide further evidence that antimicrobial use in pork production is associated with increased risk of resistance among fecal *E. coli*. In-feed antimicrobials appear to be more consistently associated with increased risk than do individual-animal treatments.

II. Introduction:

Antimicrobials are widely used in intensive food animal production for treatment and control of infectious disease and in some cases for promotion of growth and disease prevention. While there is little doubt that these drugs have for many years facilitated efficient, economical and humane food and fibre production, increasing concerns about their widespread use on farms are being voiced (1). These concerns are not new and despite the many scientific reviews that have been conducted over the years (2) there remains much uncertainty about the type and magnitude of risk to public health posed by agricultural use of these drugs. Management of any risks that do exist and maintenance of local and export markets will increasingly rely on prudent use of antimicrobials in agriculture. This is especially true in the intensive rearing systems of the North American pork industry. Understanding of the role of various antimicrobial treatment regimes in creating and maintaining resistance is needed to develop evidence-based prudent use guidelines for industry (3).

1. World Health Organization Meeting on the Medical Impact of the Use of Antimicrobial Drugs in Food Animals. October 13-17, 1997. Berlin.
2. National Research Council. The use of drugs in food animals: benefits and risks. National Academy Press. Washington, D.C., 1998.
3. Apley. M.D., et al. Task Force Report. The role of veterinary therapeutics in bacterial resistance development: animal and public health perspectives. 1998. College Park, Maryland.

III. Objectives:

From feces of pigs on farrow-to-finish operations that do or do not use antimicrobials for therapy, prevention or growth promotion:

1. Identify the relative contributions of therapeutic and in-feed antibiotics (including drug rotations) to resistance to a variety of antibiotics among *E. coli* of weaner pigs.
2. Identify the relative contributions of therapeutic and in-feed antibiotics to resistance to a variety of antibiotics among enterococci, *Salmonella*, *Campylobacter* and *Yersinia* of weaner and finisher pigs.

3. Identify temporal changes in the above resistance patterns that have occurred on study farms in the last five years.

IV. Procedures:

This study used antimicrobial treatment and antimicrobial resistance data from 32 farrow-to-finish farms located in Ontario, Canada. The rationale and method for farm selection was based on the desire to detect associations between the use of narrow-spectrum as well as broad spectrum antimicrobials and drug resistance among *E.coli* of finisher pigs. The finisher pig data have already been published, however samples were also obtained from weaner pigs and this information is the focus of the present study. Twenty individual freshly voided fecal samples from weanling (between weanling and 20 kg live weight) and finisher pigs were obtained on each of the 32 study farms in the autumn of 1992 and again in 1999. Equal volumes of faeces were composited to the farm level and were diluted in peptone water and glycerol, then frozen at –70^{o}C.

For objective 1, susceptibilities of *E.coli* in pooled samples to breakpoint concentrations of seven antimicrobials, i.e. 16 mg/ml ampicillin (Amp), 30 mg/ml carbadox (Car), 4 mg/ml gentamicin (Gen), 32 mg/ml nitrofurantoin (Nit), 16 mg/ml spectinomycin (Spe), 256 mg/ml sulfisoxazole (Sul), and 8mg/ml tetracycline (Tet), were determined. A hydrophobic grid membrane filter method was used for objective 1, because it is repeatable, accurate and capable of handling large numbers of isolates (up to 200/sample) with reasonable laboratory-resource demands. Breakpoint concentrations were based on National Committee for Clinical Laboratory Standards (NCCLS) guidelines when applicable.

For Objective 3, *E. coli* we re-isolated from the 1992 frozen samples, and 1999 samples were treated in identical fashion. Five individual lactose-fermenting colonies of bacteria with an appearance thought to be typical for *E. coli* were picked from different streak lines and plated onto MacConkey agar. These were later confirmed by a series of standard tests. The five isolates obtained from each sample were grown in Mueller- Hinton broth (Difco) and transferred by replica- plating onto Mueller- Hinton agar plates containing one of 17 antimicrobials at the following breakpoint concentrations based NCCLS guidelines when applicable: 64 □g/ml amikacin, 16 and 32 □g/ml ampicillin, 30 □g/ml carbadox, 8 □g/ml ceftiofur, 32 □g/ml cephalothin, 32 □g/ml chloramphenicol, 0.125 and 1 □g/ml ciprofloxacin, 100 □g/ml florfenicol, 4 and 16 □g/ml gentamycin, 64 □g/ml kanamycin, 32 □g/ml nalidixic acid, 64 □g/ml nitrofurantoin, 16 and 64 □g/ml spectinomycin, 32 □g/ml streptomycin, 216 and 512 □g/ml sulfisoxazole, 80 □g/ml sulfamethoxazole/trimethoprim , 8 and 16 □g/ml tetracycline.

Antimicrobial usage in 1992 was recorded as described above. Attempts were made in 1999 to re-visit all 32 farms enrolled in the 1992 study but some were no longer in business (see below). Remaining farms were contacted and visited in order to complete a questionnaire and collect fecal and feed samples. The questionnaire provided information on medication used on study farms, changes and trends in antimicrobial usage that have occurred in the last 7 years; and farmers' opinion about the antimicrobial resistance issue and other problems related to the pork industry.

Data were entered from paper records into Dbase IV or Micosoft Access files and subsequently imported into SAS, (SAS Institute, Cary, Version 6.12) data sets for statistical analysis.

Logistic regression was used to estimate the effects of antimicrobial use variables and management factors on each resistance outcome. A backward stepwise elimination procedure was used for model building purposes. A multiplicative overdispersion factor was used to adjust for all statistics, which was estimated by the division of the residual deviance by the degrees of freedom. When the antimicrobial tested had not been used on any study farm, the other antimicrobials from the same class were introduced in the model (i.e. we assumed that penicillin use on farms may select for ampicillin resistance, that the sulfamethazine use would select for sulfisoxazole resistance, that furazolidone use would select for nitrofurantoin resistance, and that spectinomycin may select not only for spectinomycin resistance, but also for streptomycin, kanamycin or gentamicin resistance and so on). For objective 2, pig type variables were: piglet (P), weanling pig (W), finisher (F), dry sow (DS), lactating sow (LS), gilt (G) and boar (B). Because of the very low numbers of isolates in some cases, which led to computational problems, piglet and boar variables were excluded and gilts were added to the dry sow category.

All farms in 1992 were purposely selected to be farrow-to-finish (FF) farms. In 1999 other management practices were in use on some of these same farms, therefore the following variables were built for statistical analysis: FF at one site, SEW (segregating early weaning) and "Other" (all the other farms, with FF production at 2 or 3 sites, farms involved in only 2 stages of growth, i.e. farrow to weaner, weaner to finisher, grower to finisher).

V. Results:

Objective 1:

Antimicrobial resistance patterns were determined for 4356 colonies of *E.coli* from the pooled fecal samples from weanling pigs on the 32 study farms. The percentage of *E.coli* resistant to each drug was estimated for each pooled fecal sample. The median, first and third quartile of the percentage of the number of *E.coli* resistant to each antimicrobial is presented in Table 1. For comparative purposes, latter percentages are listed separately for farms which did or did not routinely add antimicrobials in-feed for weanling pigs ("starter rations") for the three years prior to the data collection and for all farms. On visual inspection of the data, the percentages of resistance for each antimicrobial outcome were consistently higher on farms which added antimicrobial substances to their starter rations than of the four farms that did not.

In models for all drugs except for spectinomycin, the use of in-feed antimicrobials was significantly (p-value< 0.075) associated with the resistance outcome. Significant variables and their p-values are shown for each model in Table 2. The model building process for gentamicin could not be carried out because of convergence problems of the design matrix.

The use of antimicrobial feed-additives in starter rations was significantly and strongly associated in four of the six final logistic models with increased risk of *E.coli* resistance to the antimicrobials tested. For three of these models, however, it was not possible to estimate the

effect of the specific antimicrobial alone as it was common practice to use drugs in combination. Still, the use of antimicrobials for weanling pigs, which were almost always administered in subtherapeutic doses, was the most consistent explanatory variable across the different resistance models. This provides additional evidence that long-term and low-level exposures to antimicrobials in the gut are important risk factors increased risk of resistance.

The in-feed use of antimicrobials other than the one tested for resistance was significant in two models, in one for the addition in weanling pigs (Car) and in the other for the addition to nursing sow rations (Spc). One explanation of this is that some resistant determinants may have been transferred concurrently, most likely on the same plasmid as antibiotic resistance in *E.coli* is generally due to a high incidence of plasmid carriage. Further work would need to be done to determine if this was in fact the explanation.

In the ampicillin model, both group (i.e. in-feed use) and individual treatments of nursing sows with penicillin was associated with resistance in *E.coli*-isolates. It is conceivable that antimicrobial-contained nursing sow rations might increase the prevalence of resistance in the microbiota of weanling pigs as it is believed that maternal faeces are the major source of bacteria for new-born pigs. Individual animal treatments (of nursing sows and weanling pigs) were significant only in the final logistic model for ampicillin and only at the 6% level of significance. Penicillins were the most frequently used antimicrobial class for individual animal treatment in each class of pig on the study farms. They were given, for instance, in 61.2% of all individual treatments administered to nursing sows. This supports the notion that the percentage of resistant *E.coli* is related to the frequency with which hosts were treated with that drug and provides reasoning why individual penicillin treatments in particular appeared to be significant in this study. These results also support the current state of knowledge, i.e. short-term, full-dose therapeutic use has a smaller selective potential than low-level, long term exposure like the in-feed use of antimicrobials.

The variables describing the different farm management practices were included in this study mainly to control for possible confounding effects (e.g. herd size). The use of all-in all-out management of weanling pigs and the type of housing were each significant in two of the models (Nit, Tet, and Car, Nit, respectively) and the type of herd was associated in another (Sul). The significance in particular of the former two management characteristics might reflect a role of environmental factors in maintaining or promoting resistance. Although further research is needed to determine whether and what factors might help to decrease the resistance level on the farm, the results indicate that under certain circumstances adhering to the guidelines of “good management practice” might be beneficial.

It is particularly noteworthy that even on farms that did not add antimicrobials to their feed for the 3 years preceding data collection, the percentage of tetracycline resistant *E.coli* was greater than 75%. Thus, once antimicrobial resistant bacteria were established in these populations, the degree of resistance was apparently not readily reversed by restrictive usage of antimicrobials.

Objective 2:

No *Salmonella* were recovered from any of the fecal samples collected in 1992 and 1999 and in view of the high costs of farm visits and the more productive *E. coli* studies described for objectives 1&2, Campylobacter and Yersinia tests were not undertaken. Isolations of enterococci have been made but financial resources provided by this grant were not sufficient to cover the costs of resistance testing for these organisms which is much more expensive and technically demanding than was foreseen at the time of proposal writing. This testing is now being conducted in the lab of a research collaborator, Dr. Case Poppe, with the aid of funding from another source. NPPC will be credited with a valuable contribution to the enterococci work when it is completed later this year.

Objective 3:

In 1992, 32 farrow-to-finish farms were enlisted, and by 1999, 15 were still farrow-to-finish farms with production on one site, 5 of the farms went out of business, 4 became SEW, 6 had changed management structure (to 2 or 3 site production, or involved in only 2 stages of growth), 2 did not allow a follow-up visit. These changes were accompanied by variations in herd size.

The most commonly used in-feed antimicrobials in both 1992 and 1999, were tetracycline, penicillin, sulfamethazine, tylosin and carbadox, while some antimicrobials used in 1992 were no longer used in 1999, i.e. furazolidone, salinomycin, streptomycin and zinc bacitracin. Most in-feed administrations of antimicrobials were offered in piglet and weaner rations, accounting for approximately 2/3 of total administrations. Penicillin was used widely in the sample farms for individual treatments, accounting for more than one third of total individual treatments both in 1992 and 1999. Next in importance as antimicrobials used for individual treatment were tylosin, tetracycline, trimethoprim and sulfadoxine. The major change in the usage of drugs for individual treatment was the withdrawal of streptomycin, which was used in all pig categories in 1992 and in none of them in 1999. Antimicrobials were only occasionally added to water in the study farms, most commonly lincomycin and spectinomycin and more recently apramycin.

Nine hundred *E. coli* isolates were tested for antimicrobial resistance and the prevalence from each pig category in 1992 and 1999 is presented in detail in Table 3. Figure 1 shows that prevalences are higher in 1999 for all drugs except for carbadox, nitrofurantoin, streptomycin and sulfamethoxazole/trimethoprim.

All *E coli* strains isolated were susceptible to four of the 17 antimicrobials tested: amikacin, ciprofloxacin at 1 and 0.125 µg/ml, florfenicol, and nalidixic acid. No *E coli* isolates were resistant to ceftiofur in 1992, however there was one isolate resistant in 1999. Regression models were not built for these antimicrobials given the lack of resistance observed. The regression model for gentamicin resistance could not be carried out because of convergence problems of the design matrix.

In 10 out of the 15 final regression models, in-feed administration of antimicrobials was significantly associated with increased risk for resistance. The use of penicillin was associated with increased risk of resistance to ampicillin ($p< 0.01$), carbadox use was associated with carbadox resistance ($p< 0.01$), furazolidone use with nitrofurantoin resistance ($p< 0.05$), spectinomycin use with spectinomycin resistance ($p< 0.05$) and with kanamycin resistance ($p<$

0.05), sulfamethazine use with increased resistance to sulfisoxazole ($p< 0.01$) and tetracycline use with tetracycline resistance ($p< 0.05$). Individual treatment was significant in only one regression model (streptomycin). In-water administration of spectinomycin was significant in the final regression model of kanamycin ($p< 0.01$) and had a negative effect on the resistance outcome.

The prevalence of resistance increased in 14 out of the 23 pairs of prevalences analyzed. Nevertheless, only in 4 regression models were increases statistically significant: cephalothin ($p< 0.05$), chloramphenicol ($p< 0.05$), tetracycline at 8 and 16 □g/ml ($p< 0.05$). Four resistance prevalences decreased, but only one decreased significantly: nitrofurantoin ($p< 0.01$).

The use of antimicrobial feed additives in pigs' rations was significantly associated in ten of the fifteen final logistic models with increased risk of *E. coli* resistance to the antimicrobials tested, being by far the most important factor among the ones included in the regression models. In-feed administration is the most likely to select for antimicrobial resistance because the drug concentration is low enough to allow bacteria to grow at some level, but high enough to exert a selective pressure and because all pigs in a group are exposed, in some cases for long periods of time.

We found a significant decrease in nitrofurantoin resistance from 1992 to 1999. Furazolidone was used in feed in 1992, in piglets and weanling pigs, but it was withdrawn in Canada in 1994 because of the concerns that the drug might have carcinogenic potential. This temporal association adds further evidence of a causal relationship between in-feed use and resistance and it offers some hope that reduction in usage of other drugs could reverse the resistant status of bacteria.

The change in streptomycin resistance pattern is another example where the resistance prevalence decreased consequent to the cessation of use. In 1992, streptomycin was used mostly for individual treatment and because of its long withholding period and concerns over residues, it was taken out of many injectable penicillin products. This was followed by a reduction in prevalence seen in 1999.
All strains of *E. coli* were sensitive to amikacin, florfenicol, ciprofloxacin and nalidixic acid. The lack of resistance to fluoroquinolones is especially encouraging given the fact that they are currently the drugs-of-choice for invasive food borne infections in humans. Fluoroquinolones are not licensed in Canada for use in pig production.

Resistance to tetracycline was high on nearly all farms and for all phases of growth. High incidence of *E. coli* resistance against tetracycline is a common finding worldwide where these antibiotics are included in animal feeds. Resistance to tetracycline is not only widespread, but also very stable.

Chloramphenicol resistance has increased significantly although it is not approved for use in food producing animals in Canada. In our study we found that chloramphenicol resistance was correlated with resistance to tetracycline (OR= 9.53), ampicillin (OR= 5.55), and sulfisoxazole (OR= 46.19), and this might support the idea of a linkage of resistance determinants, although characterization studies are needed to answer this question. The possibility of co-selection by other drugs should be assessed in future research.

Conclusions:

Objective 1: The in-feed addition of a penicillin, carbadox, a sulphonamide and a tetracycline to weanling pig rations ("starter rations") was associated with increased risk of resistance to ampicillin, carbadox, sulfisoxazole and tetracycline, respectively. Individual weanling pig treatments were significant only in the ampicillin model. Management and housing factors were also significantly associated with the risk of resistance but without any consistent pattern. These results indicate that antimicrobial exposure to groups of weanling pigs through feed is more consistently associated with increased risk of resistance among *E. coli* than individual animal treatment.

Objective 3: Our study shows that for antimicrobials that were consistently used as in-feed additives during the study interval, the resistance prevalence increased or remained at a steady level, while for some of the drugs that have been discontinued, this prevalence decreased, most notably in the case of nitrofurantoin.

These studies provide further evidence that antimicrobial use in pork production is associated with increased risk of resistance among fecal *E. coli*. In-feed antimicrobials appear to be more consistently associated with resistance increases than do individual-animal treatments. Pork producers and their veterinarians need to be constantly vigilant that antimicrobials are used judiciously, particularly in the case of in-feed medications, so that these valuable drugs continue to be effective for treatment of animal and human infections in the future.

Table 1 : Percentages of faecal *E.coli* from weanling pigs resistant to antimicrobials on 32 farrow-to finish farms (1992)

Antimicrobial	***Percent of* E.coli *resistant to antimicrobial***								
	No antimicrobials in starter rations (n=4 farms)			Antimicrobials in starter rations (n=28 farms)			***All farms*** (n=32 farms)		
	Lower Quartile	Median	Upper quartile	Lower quartile	Median	Upper quartile	Lower quartile	Median	Upper Quartile
Ampicillin	0.7	7.9	45.2	11.5	38.3	90.7	8.7	31.2	88.5
Carbadox	0	0.3	0.6	0	2.3	31.8	0	1.2	24.4
Gentamicin *	0	0	0	0	0.78	0	0	0.7	0
Nitrofurantoin	4.99	23.8	40.9	15.1	33	63.7	12.4	33.0	56.5
Spectinomycin	14.8	32.4	49.7	19.7	45.6	75.2	19.7	39.1	72.7
Sulfisoxazole	14.4	32.9	54.93	23.3	55.1	79.2	23.4	52.6	77.3
Tetracycline	73.21	76.8	86.6	91.06	97.1	99.2	89.2	96.8	98.9

* means were used for gentamicin because all medians were zero.

Table 2: Final logistic regression models[a] of antimicrobial resistance in a study of 32 farrow-to-finish farms

	Model	Significant variables	p-value	Odds Ratio	Confidence Interval	
					lower limit	upper limit
VI.	**Ampicillin**	In-feed use of Pen in nursing sows	3.E-04	88.01	5.39	$2.12*10^7$
		In-feed use of Pen in weanling pigs	0.016	12.36	1.55	147.17
		Individual treatment of nursing sows with Pen	0.061	4.57	0.94	28.41
		Individual treatment of weanling pigs with Pen	0.064	2.93	0.94	9.90
Carbadox		In-feed use of Car in weanling pigs	1E.-04	42.91	9.11	312.44
		In-feed use of "other" antimicrobials in weanling pigs	9E.-04	12.13	2.52	96.72
		Type of Housing[b] for finishers (overall)	0.042			
		- Barn attached	0.030[c]	6.68	1.41	48.92
		- Bankbarn	0.404[c]	3.06	0.15	41.93
Nitrofurantoin		In-feed use of Nit in piglets	3.E.-04	72.33	5.80	$5.67*10^3$
		All-in all-out management of weanling pigs	2.E.-04	0.20	0.02	0.71

For more information contact:
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	Type of Housing [b] for finishers (overall)	0.029			
VII.	**- Barn attached**	0.017 [c]	3.89	1.34	12.66
VIII.	**- Bank barn**	0.643 [c]	1.42	0.31	6.23

Table 2: Final logistic regression models[a] of antimicrobial resistance in a study of 32 farrow-to-finish farms

Model	Significant variables	p-value	Odds Ratio	Confidence Interval	
				lower limit	upper limit
Spectinomycin	In-feed use of "other" antimicrobials than Spc in nursing sows	0.002	7.727	4.84	12.591
Sulfasoxizole	In-feed use of Sul in weanling pigs	0.074	4.83	0.86	30.86
	Type of herd[d] (overall)	0.031		/	/
	Type of herd: Conventional	0.016 [c]	9.00	1.70	67.24
	Type of herd: Semi-closed	0.116 [c]	4.72	0.80	39.41
Tetracycline	In-feed use of Tet in weanling pigs	6.E-05	17.51	4.22	95.68
	Use of all-in all-out management of weanling pigs	5.E-05	0.17	0.07	0.41

[a] model building for the proportion of gentamicin-resistant *E.coli* was not performed because of convergence problems of the design matrix

[b] baseline level: grower-finisher barn separated

[c] Wald's Standard error

[d] baseline level: closed herd

Table 3: The prevalence of antimicrobial resistance in fecal *E.coli* from each category in 1992 and 1999

Anti-biotic	Resistant isolates																								
	Total					Piglet		Weaner				Finisher				Dry sow				Lactating sow			Boars		
	'92		'99		'92	'99		'92		'99		'92		'99		'92		'99		'92	'99		'92		'99
	n	%	n	%	-	n	%	n	%	n	%	n	%	n	%	n	%	n	%	-	n	%	n	%	-
Ami 32	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	-
Amp 16	257	40.5	143	53.9	-	0	0	136	44.6	74	70.5	120	38.7	52	52	1	10	5	16.7	-	12	48	0	0	-
Amp 32	254	40	143	53.9	-	0	0	134	43.9	74	70.5	119	38.4	52	52	1	10	5	16.7	-	12	48	0	0	-
Carb 30	63	9.92	25	9.43	-	0	0	50	16.4	18	17.1	13	4.19	7	7	0	0	0	0	-	0	0	0	0	-
Cef 8	0	0	1	0.38	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	1	4	0	0	-
Cep 32	22	3.46	16	6.04	-	0	0	11	3.61	11	10.5	10	3.23	3	3	0	0	1	3.33	-	1	4	1	10	-
Chl 32	29	4.47	26	9.81	-	0	0	23	7.54	18	17.1	6	1.94	5	5	0	0	1	3.33	-	2	8	0	0	-
Cip 0.125	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	-
Cip 1	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	-
Flo 100	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	-
Gen 4	14	2.2	30	11.3	-	0	0	9	2.95	16	15.2	5	1.61	14	14	0	0	0	0	-	0	0	0	0	-
Gen 16	11	1.73	9	3.4	-	0	0	8	2.62	0	0	3	0.97	9	9	0	0	0	0	-	0	0	0	0	-

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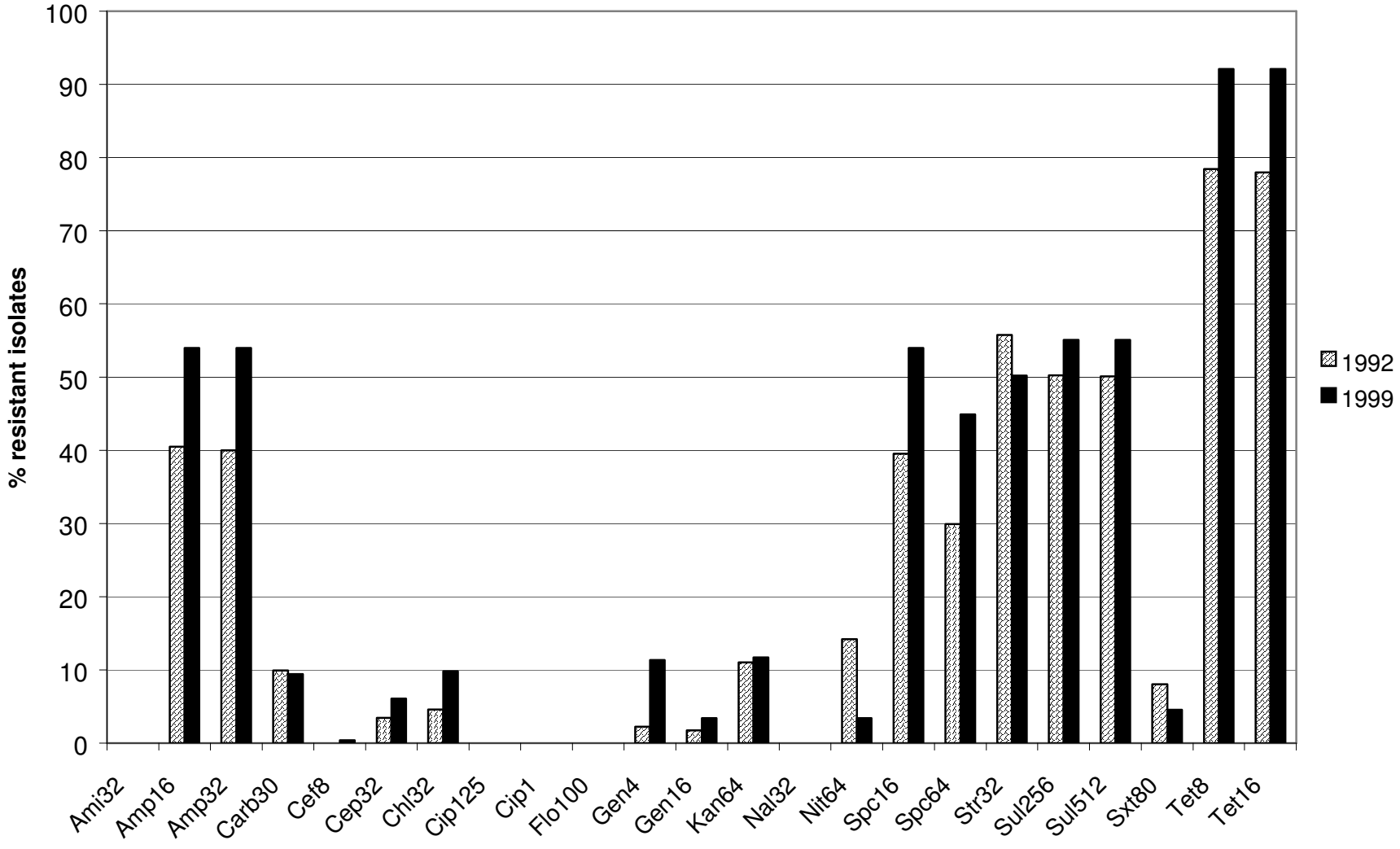
Kan 64	70	11.0	31	11.7	-	0	0	41	13.4	16	15.2	29	9.35	8	8	0	0	2	6.67	-	5	20	0	0	-
Nal 32	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	-
Nit 64	90	14.2	9	3.4	-	0	0	42	13.8	5	4.76	48	15.5	0	0	0	0	1	3.33	-	3	12	0	0	-
Spc 16	251	39.5	143	53.9	-	2	40	150	49.2	53	50.5	101	32.6	60	60	0	0	16	53.3	-	12	48	0	0	-
Spc 64	190	29.9	119	44.9	-	2	40	117	38.4	48	45.7	73	23.5	45	45	0	0	12	40	-	12	48	0	0	-
Str 32	354	55.7	133	50.1	-	1	20	186	61	49	46.7	166	53.5	54	54	1	10	17	56.7	-	12	48	1	10	-
Sul 256	319	50.2	146	55.0	-	0	0	169	55.4	69	65.7	149	48.1	55	55	0	0	11	36.7	-	11	44	1	10	-
Sul 512	318	50.1	146	55.0	-	0	0	167	54.7	69	65.7	150	48.4	55	55	0	0	11	36.7	-	11	44	1	10	-
Sxt 80	51	8.03	12	4.53	-	0	0	29	9.51	4	3.81	22	7.1	4	4	0	0	1	3.33	-	3	12	0	0	-
Tet 8	498	78.4	244	92.1	-	5	100	256	83.9	97	92.4	229	73.9	94	94	7	70	27	90	-	21	84	6	60	-
Tet 16	495	77.9	244	92.1	-	5	100	256	83.9	97	92.4	227	73.2	94	94	6	60	27	90	-	21	84	6	60	-

Figure 1: Antibiotic resistance patterns of *E.coli* from swine feces in 1992 vs 1999



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