

Some Characteristics and Antibiotic Resistance of Anaerobic Bacteria from the Ceca and Colons of Pigs Fed Chlortetracycline-Containing and Unmedicated Diets†

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Morphology and end product patterns were used to group 112 bacterial isolates from the ceca and colons of pigs fed unmedicated and chlortetracycline-containing diets. Most of the isolates (68%) were resistant to chlortetracycline ($>4 \mu\text{g/ml}$). Chlortetracycline resistance was associated with all groups of anaerobic and facultative bacteria.

Antibiotics are commonly used as growth promoters in animal feeds in the United States. The mechanism by which these agents influence animal growth is not understood, but it is commonly believed that the growth-promoting activities can be explained by the effects of antibiotics on the gastrointestinal microflora (11). Increased awareness of antibiotic resistance and the potential health hazards associated with the continued use of these drugs has made their use controversial. However, few studies have examined the effects of growth-promoting antibiotics on the predominant anaerobic bacteria in the intestinal tract. Surveys have shown that antibiotic resistance in gram-negative anaerobes is greater in the feces of swine fed chlortetracycline (CTC) (1) and suggest that a wide variety of bacterial groups in fecal material can be resistant to CTC (12).

Previous studies in our laboratory have used strict anaerobic techniques to show that oral administration of CTC can increase the proportions of CTC-resistant bacteria in the predominantly anaerobic bacterial population in the ceca and colons of pigs from a herd which was not receiving antibiotics (K. A. Dawson, B. E. Langlois, T. S. Stahly, and G. L. Cromwell, *Abstr. Annu. Meet. Am. Soc. Anim. Sci.* 1981, 282, p. 238). The purpose of the present study was to examine some of the characteristics and CTC resistance of representative anaerobic bacterial isolates from the ceca and colons of pigs fed an unmedicated diet or a diet containing either a subtherapeutic (27.5 $\mu\text{g/g}$) or a therapeutic (220 $\mu\text{g/g}$) concentration of CTC.

Pigs were obtained from two herds with different histories of antibiotic exposure. One group of pigs was from our nonantibiotic (NAB) herd, which had not received antibiotics in feed or therapeutically for 8 years at the time of this study (7). The second group of pigs was from our antibiotic (AB) herd, which routinely received CTC in feed. All pigs used in this study were fed an unmedicated corn-soybean meal diet before dietary treatments were initiated. All pigs were housed in a common production facility at 7 to 9 weeks of age. Pigs from each herd were allowed a 21-day adjustment period and then were assigned to one of the three dietary treatment groups.

After 2 weeks of treatment, two pigs from each control and treatment group were slaughtered. Immediately after

death, segments (approximately 25 g of tissue and contents) of the cecum, proximal colon, and distal colon of each pig were ligated, removed into blender jars, and transported into an anaerobic chamber. Each segment was ground and blended with anaerobic dilution solution (5). Serial dilutions of the blended material were used to inoculate roll tubes containing CCA medium (2) that had been prepared with energy-depleted rumen fluid. All media and cultures were prepared under a CO_2 gas phase by a modification of the Hungate technique to maintain strict anaerobic conditions (4).

A total of 144 isolates (12 per pig) were randomly picked from roll tubes containing between 10 and 100 colonies. These tubes represented between 10^{-8} and 10^{-7} g of intestinal material. Only 112 of these isolates were successfully maintained in pure culture on CCA slants. Isolates represented organisms from all three segments of the intestinal tract. Each of these isolates was partially characterized and identified according to Gram-stain reaction, form, and end product formation from glucose, by procedures outlined by Holdeman et al. (6).

Table 1 shows the presumptive identification of isolates from the ceca and colons of pigs from all treatment groups. Nineteen percent of the isolates were facultative anaerobes. Facultative isolates were associated with the streptococcus and lactobacillus groups. Sixty-two percent of the isolates obtained from the ceca and colons were gram positive. This predominance of gram-positive organisms is consistent with the other studies of intestinal (9) and fecal (10) anaerobes from pigs. However, our results from studies of isolates obtained from the ceca and colons tended to be quantitatively different from those of Robinson et al. (8), who isolated relatively few lactobacilli and streptococci from the ceca of pigs by using a similar habitat-simulating medium.

The susceptibility of isolates to CTC was tested in an anaerobic chamber by using the agar dilution technique described by Brown and Waatti (3) and a modified replicator that inoculated each plate with 0.05 ml of an appropriate dilution of cell suspension. Isolates that would not grow on plates in the anaerobic chamber were tested for their susceptibility to CTC in peptone-yeast extract-glucose broth (6) supplemented with 5% clarified rumen fluid. Antibiotic susceptibilities were measured by comparing the growth of isolates in antibiotic-containing media with growth in antibiotic-free media.

More than 65% of the isolates from pigs in all groups were resistant to CTC at a concentration greater than 4 $\mu\text{g/ml}$

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TABLE 1. Presumptive identifications of predominant groups of bacteria from the ceca and colons of swine

Bacterial group	Gram-stain reaction	Form	Acid from glucose ^a	Products from glucose ^b		Tentative identification (genus)	Isolates ^c	
				Major	Minor		No.	%
Lactobacilli	+	Rods	+	L	(A)	<i>Lactobacillus</i>	32	28.5
Bacteroids	-	Rods	+	AS	(P)	<i>Bacteroides</i>	30	26.8
Streptococci	+	Cocci	+	L	(AP)	<i>Streptococcus</i>	16	14.3
Nonfermentative rods	+	Rods	-		(ASLBP)	<i>Eubacterium, Clostridium</i>	12	10.7
Fermentative rods	+	Rods	+	AL(P)	(S)	<i>Eubacterium, Bifidobacterium, Propionibacterium</i>	9	8.4
Selenomonads	-	Curved rods	+	AP(LS)		<i>Selenomonas</i>	7	6.2
Other cocci	-	Cocci	- (+) ^d	(ABC)	(PSI)	<i>Ruminococcus, Megasphaera, Acidaminococcus, Veillonella</i>	6	5.3

^a Acid production from glucose was measured in peptone-yeast extract-glucose medium (6). pH values of less than 6.2 after 24 h were considered positive.

^b Abbreviations: L, lactic acid; A, acetic acid; B, butyric acid; P, propionic acid; S, succinic acid; C, caproic acid; I, isovaleric acid. Major products were produced in amounts of 10 μ mol/ml or greater; minor products were produced in amounts less than 10 μ mol/ml. Products in parentheses were formed by only a few strains in each group.

^c Data from 112 isolates obtained from 12 pigs.

^d Only a few strains produced acid from glucose.

TABLE 2. Minimal inhibitory concentrations of CTC for the predominant groups of bacteria from the ceca and colons of swine^a

Bacterial group	% of isolates inhibited by CTC at concn (μ g/ml):		
	≤ 4	8-64	≥ 128
Lactobacilli	12.5	31.2	56.3
Bacteroids	20.0	73.3	6.7
Streptococci	81.3	18.7	0
Nonfermentative rods	33.3	58.3	8.3
Fermentative rods	22.2	33.3	44.4
Selenomonads	71.4	28.6	0
Other cocci	33.3	33.3	33.3
All groups	32.1	43.8	24.1

^a Data from 112 isolates obtained from 12 pigs.

(Table 2). These proportions of resistant organisms tended to be greater than those observed in other studies of anaerobic bacteria from pig feces (1, 12). CTC-resistant organisms were found in all bacterial groups. A majority of the lactobacilli, bacteroids, nonfermentative rods, and fermentative rods were resistant to either high or intermediate levels of

CTC, whereas streptococci and selenomonads tended to be susceptible to CTC at concentrations greater than 4 μ g/ml. This distribution of resistant organisms is considerably different from that described by others. Welch and Forsberg (12) isolated relatively few CTC-resistant lactobacilli and bacteroids and found that 34% of the CTC-resistant isolates in the feces of swine fed CTC were streptococci.

The distribution of the different bacterial groups among the control and treatment groups is shown in Table 3. Lactobacilli consistently made up a greater proportion of the microbial population in AB pigs than in NAB pigs. However, CTC-resistant lactobacilli were isolated from pigs in all groups. Despite the fact that lactobacilli tended to be resistant to a greater concentration of CTC than any other bacterial group could resist, the proportion of lactobacilli in the total population was consistently lower when CTC was included in the diet. The proportions of the other bacterial groups were not consistently altered by antibiotic treatment.

The proportion of CTC-resistant organisms was consistently lower in NAB pigs than in AB pigs (Table 3), even though no attempt was made to isolate these pigs within the production facility. In addition, the proportion of CTC-resistant organisms was greater in pigs receiving therapeutic and subtherapeutic CTC than in the controls. These results

TABLE 3. Distribution of predominant bacterial groups among control and treatment groups of pigs

Bacterial group	% of isolates in group ^a :							
	NAB pigs				AB pigs			
	Control (18) ^b	Subtherapeutic CTC (18)	Therapeutic CTC (17)	Total (53)	Control (20)	Subtherapeutic CTC (21)	Therapeutic CTC (18)	Total (59)
Lactobacilli	22	17	18	19	45	38	28	37
Bacteroids	39	22	23	29	25	14	39	25
Streptococci	6	6	29	15	10	19	11	14
Nonfermentative rods	22	6	6	11	5	19	6	10
Fermentative rods	0	22	12	11	10	0	6	5
Selenomonads	0	22	0	8	5	5	6	7
Other cocci	11	0	6	8	0	0	11	3
CTC resistant ^c	50	56	59	55	70	76	89	78

^a NAB pigs had not been exposed to antimicrobial agents for 8 years; AB pigs routinely received CTC in feed. Pigs in control groups received an unmedicated diet; others received diets containing subtherapeutic (27.5 μ g/g) or therapeutic (220 μ g/g) amounts of CTC.

^b Numbers in parentheses show number of isolates identified in each group.

^c Percentage of isolates resistant to CTC concentrations greater than 4 μ g/ml.

are consistent with studies of CTC-resistant bacteria in pig feces (7, 11).

The results of this study indicate that CTC resistance can be associated with a wide variety of anaerobic bacteria. However, the presence of a high proportion of CTC-resistant organisms in a particular bacterial group did not give that group a competitive advantage when CTC was included in the feed. Complex anaerobic populations were maintained in the ceca and colons regardless of antibiotic treatment.

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