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Short communication

Antimicrobial susceptibility patterns of competitive exclusion bacteria applied to newly hatched chickens

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Abstract

Competitive exclusion (CE) products are mixtures of obligate and facultative anaerobic bacteria applied to poultry hatchlings for prevention of *Salmonella* colonization. These mixtures have the potential to introduce bacteria with undesirable antimicrobial drug resistance traits into the human food supply. Antimicrobial drug susceptibilities of 27 obligate and facultative anaerobes isolated from a commercial CE product were evaluated with a microdilution minimal inhibitory concentration (MIC) assay. *Bacteroides distasonis* and *Bacteroides fragilis* isolates were resistant to tetracycline and other antimicrobial drugs. An *Escherichia coli* isolate was resistant to four antimicrobial drugs: erythromycin, penicillin, vancomycin, and tylosin. Erythromycin-resistant enterococci and vancomycin-resistant *Lactococcus lactis* isolates in the CE product were detected. These findings suggest that more work needs to be done to assess the potential effects of CE product use in poultry on the food supply.

Keywords: Competitive exclusion; Antimicrobial susceptibility testing; Anaerobic; Poultry; Antimicrobial drug resistance

1. Introduction

Competitive exclusion products are cultured mixtures of intestinal bacteria from healthy adult chickens produced commercially (Nisbet et al., 1995) for application on newly hatched chicks. Complex mixtures containing a diversity of bacteria are reported to be more effective than single species or simple defined bacterial mixtures for protection of chicks from intestinal colonization by *Salmonella* (Mead and Impey, 1987; Stavric and D'Aoust, 1993). Defined mixtures of bacteria are seen to be less effective as CE agents and have shorter shelf lives than the undefined mixtures isolated and cultured from intestinal contents of adult chickens (Hofacre et al., 2000). The incomplete definition of CE products leaves the possibility that these bacteria could have antimicrobial drug resistance determinants that are transferable from the poultry microbiota to the intestinal microbiota of the human consumer (Kruse, 1999), which can lead to life-threatening infections in susceptible individuals (Falk et al., 1998). The application of a bacteria consortium

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from one flock of birds to a large population of hatchlings that are distributed to many farms has the potential to spread antimicrobial drug resistance widespread into the human food supply via poultry.

Food animals have been identified as a source of antimicrobial-resistant bacteria and resistance determinants for transfer to human intestinal bacteria (Van den Bogaard and Stobberingh, 2000; Oppegaard et al., 2001). Antimicrobial drug-resistant bacteria have been isolated from poultry litter, suggesting that it could be an environmental reservoir for resistance (Kelly et al., 1998). Streptothricin resistance transfer in *Escherichia coli* and vancomycin resistance transfer in enterococci are examples of food-borne transfer of antimicrobial drug resistance to human intestinal flora (Witte, 2000). In fact, poultry have been recognized as a source for vancomycin-resistant *Enterococcus faecium* (Wegener et al., 1997).

In the present study, a microdilution MIC assay was used to determine antimicrobial resistance patterns in bacteria isolated from a CE product to the following antimicrobials: erythromycin, nalidixic acid, penicillin, tetracycline, vancomycin, ciprofloxacin, and tylosin. Vancomycin is the human-use analog of avoparcin, formerly used as a growth promoter for poultry in Europe (Van den Bogaard and Stobberingh, 2000; Wegener et al., 1997).

2. Materials and methods

2.1. Material sources

Control bacteria (*Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29711, and

Table 1
Antimicrobial susceptibilities of isolates from a commercial competitive exclusion product

Isolate	Minimal inhibitory concentration (μg/ml)						
	Eryth	NA	Pen	Tet	Van	Cipro	Tylosin
Bacteroides fragilis 1	2	32	256	0.25	1	4	0.25
Bacteroides fragilis 2	16	8	>256	1	4	0.5	0.25
Bacteroides fragilis 3	>256	64	>256	16	256	0.25	0.25
Bacteroides fragilis 4	256	64	>256	2	16	4	256
Bacteroides distasonis 1	256	128	>256	64	>256	16	>256
Bacteroides distasonis 2	>256	64	>256	128	>256	>256	>256
Acinetobacter baumannii 1	>256	0.5	32	0.25	8	0.25	8
Acinetobacter baumannii 2	256	0.5	16	0.5	4–8	0.25	8
Stenotrophomonas maltophilia	>256	1	>256	0.5	8	1	128
Escherichia coli	>256	0.5	125	<0.125	16	<0.125	64
Enterococcus faecalis 1	>256	64	256	1	1	2	2
Enterococcus faecalis 2	32	>256	0.25	<0.125	0.5	2	0.5
Enterococcus faecalis 3	0.5	64	0.5	0.25	0.25	0.125	1
Enterococcus faecalis 4	32	32	0.25	0.25	1	0.5	0.5
Enterococcus avium 1	256	32	<0.125	4	0.25	0.25	0.25
Enterococcus avium 2	32	>256	0.25	4	0.125	0.125	0.25
Enterococcus avium 3	128	16	<0.125	4	0.5	0.25	1
Lactococcus lactis 1	>256	32	>256	128	256	>256	>256
Lactococcus lactis 2	>256	64	>256	128	128	128	>256
Pediococcus parvula	64	64	<0.125	0.5	>256	2	0.25
Lactobacillus salivarius 1	128	32	>256	<0.125	256	<0.125	<0.125
Lactobacillus salivarius 2	128	8	<0.125	1	0.5	<0.125	0.25
Lactobacillus salivarius 3	>256	256	<0.125	>256	256	0.5	<0.125
Lactobacillus salivarius 4	256	64	0.25	1	>256	2	0.125
Lactobacillus salivarius 5	>256	128	>256	16	>256	>256	256
Lactobacillus salivarius 6	256	64	<0.125	4	>256	2	0.5
Lactobacillus ruminus	32	64	<0.125	16	>256	0.25	0.25

Abbreviations: Eryth=erythromycin, NA=nalidixic acid, Pen=penicillin, Tet=tetracycline, Van=vancomycin, Cipro=ciprofloxacin.
Interpretive standards for Eryth, NA, Pen, Tet, Van, Cipro, and Tylosin, respectively are: sensitive ≤0.5, ≤8, ≤8, ≤4, ≤4, ≤1, ≤0.5; resistant ≥8, ≥32, ≥16, ≥16, ≥32, ≥4, ≥8.