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GLYCOPEPTIDES SUSCEPTIBILITY AMONG ENTEROCOCCI ISOLATED FROM A POULTRY FARM IN SÃO PAULO, BRAZIL (1996/1997)

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ABSTRACT

Enterococci resistance to glycopeptides was evaluated em Brazilian poultry fed with feed supplemented with avoparcin as growth promoter. The susceptibility to the glycopeptides avoparcin, teicoplanin and vancomycin was determined for 217 enterococci isolated from cloacal swabs (one swab per bird) in tests and controls groups. Tests group comprised three groups (A, B and C) of Hubbard broiler-chickens 14, 21 and 35 days old, respectively. These birds were from one single farm, with a common feed source supplemented with avoparcin (10 mg/kg of feed). Controls groups (1 and 2) comprised 25 and 42 days old broilers, respectively, obtained from the Faculty of Veterinary Medicine's aviary (University of São Paulo) where avoparcin was never used. No glycopeptide resistant enterococci strain was found, but an increase of *Enterococcus faecium* in faeces of chickens fed with avoparcin, independent of the age of the bird, was detected.

Key words: glycopeptides, avoparcin, vancomycin, resistance, *Enterococcus*

INTRODUCTION

Enterococci are receiving increased attention because their role in serious human hospital infections (20, 26). Reports from Europe about vancomycin-resistant enterococci (VRE) correlate this resistance with the use of the vancomycin-like glycopeptide avoparcin as growth promoter in the feed for poultry and pigs.

The extralabel uses of antibiotics in food-producing animals can increase the level of drug-resistant zoonotic pathogens in treated animals (3, 5, 6, 7, 11, 12, 14, 17, 32). Antimicrobial resistant enterococci can be transmitted to humans through contact with farm animals and through the environment (1, 12, 26). There are reports from Europe on occupationally acquired vancomycin resistant *Enterococcus faecalis* infection from poultry fed with feed supplemented with avoparcin (1, 2, 4, 9, 10, 22, 24).

Once no data is available about vancomycin resistant enterococci strains in Brazilian poultry farms we evaluated the susceptiblity of enterococci isolated from broilers chickens to the glycopeptides avoparcin, teicoplanin and vancomycin.

The objective of this study was to investigate the presence of VRE in a São Paulo poultry farm.

MATERIALS AND METHODS

Faecal samples

In 1996-1997 period, cloacal swab samples were collected from Hubbard broiler-chickens fed with feed suplemented or not with avoparcin. One cloacal swab was obtained from each bird, in both test and control groups. Test groups were obtained in a poultry farm near to São Paulo city, Brazil. This farm has several aviaries, each one with birds in a different stage of growth. The criteria for inclusion of this farm in the study was the introduction of feed containing avoparcin. Test groups A, B and C comprised aviaries with 14, 21 and 35 days old birds, respectivelly. Each aviary had 5,000 to 8,000 birds and the chickens were selected aleatorily. The birds of test groups received feed supplemented with avoparcin from the first day

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until the 35th day of life (10 mg/kg feed). The control groups were obtained at the aviary of the Faculty of Veterinary Medicine, University of São Paulo, where avoparcin was never used. The birds from Control groups 1 and 2 were 25 and 42 days old, respectivelly. It was not possible to obtain a 14 days old control group. Details and characteristics of the sources and enterococci isolated in each source are shown in Tables 1 and 2.

Microbiology

Each faecal sample was inoculated into a tube with 0.7 % nutrient agar (Nutrient agar, Difco, Detroit, MI, USA). Samples were transported to the microbiology laboratory of the Faculty of Veterinary Medicine, University of São Paulo, and, within 2-4h, direct plated onto blood-agar plates (Columbia Blood Agar, Difco, Detroit, Mi, USA) and onto blood agar plates supplemented with 5% sheep blood and 300 U/ml of polimixin B (Roerig-Pfizer, manufactured by The Upjohn Company, Kalamazoo, Michigan, USA) (23, 25).

Pure cultures were submitted to Gram stain, catalase, 6.5% NaCl and bile-aesculin tests (13, 15, 16). The partially identified strains were frozen in 10% skim milk at -20ºC for further species identification. Three or four colonies from each suspicious sample, identified as *Enterococcus* sp, were selected for identification at the species level by Facklam and Sahm method (16): tolerance to bile, growth in 6.5% salt, fermentation of pyruvate, pigment production, haemolysis in sheep blood agar, carbohydrates fermentation (mannitol, sorbitol, sorbose, arabinose, raffinose and sucrose), arginin hydrolysis, 0.04% telurite tolerance and motility. Cultures were monitored for yellow pigmentation on cotton swabs used to pick up growth from BHI plates incubated overnight. Haemolysis (alpha, beta, non-haemolysis) was determined by observation of 24-h growth on a blood-agar plate (Columbia Blood Agar 5% sheep blood Difco). Inoculum preparation for the conventional tests of identification was done on blood-agar plates.

Susceptibility tests

A screening method was performed to detect vancomycin resistance by an agar-dilution method in brain-heart infusion agar (BHIA, Oxoid, Basingstoke, Hampshire, England) with 6 µg/ml of vancomycin according to National Committee for Clinical Laboratory Standards (NCCLS) (29, 30).

Minimum inhibitory concentrations (MIC) of vancomicin hydrochloride (Europharma, Korea), teicoplanin (Lepetit, Italy), and avoparcin sulphate analytical standard (Roche, England) were determined using the standard agar dilution method, according to NCCLS (29, 30, 32). Miller Hinton Agar (Oxoid, Basingstoke, Hampshire, England), spot inoculated with approximately 10^4 CFU, was used.

For quality-control tests, vancomycin-susceptible *Enterococcus faecalis* ATCC 29212 and vancomycin-resistant ATCC 51299 (VanB phenotype) were used (31).

RESULTS

A total of 217 isolates was obtained from 165 samples (Tables 1 and 2). The MIC results for avoparcin, teicoplanin and vancomycin in both Test and Control groups are shown in Table 3. No isolate presented glicopeptide resistance.

The impact of avoparcin on the microbiota of birds of test and control groups, according to the age, was compared. A prevalence of *E. faecium* in the test groups, independent of the age of bird, was detected (Table 2). *E. faecium* was detected in 52.8% of birds of the Test groups, but only in 11.9% of the control groups.

DISCUSSION

By December 1995, there were no publications or reports on vancomycin resistant enterococccci in Brazil, on both veterinary or clinical areas. The first vancomycin-resistant enterococci (*E. faecium)* with *van*A phenotype was isolated in 1997 in a Brazilian human hospital (33, 34).

In spite of the prohibition of extralabel use of avoparcin in the USA and various European countries, its use as feed additive was unrestricted in Brazil until 1998, when the extralabel use of avoparcin was also prohibited.

Enterococci are part of the gut microbiota in both humans and birds. The veterinary use of avoparcin may therefore provide a selective pressure for the emergence of glycopeptide resistance in enterococci colonized farm animals. The avoparcin selective pressure was withdrawn from the Brazilian agriculture in 1998, but the use of glicopeptides vancomycin and teicoplanin is continuous in hospitals.

With respect to the frequent use of vancomicin in hospitals, similar situation occurs in Brazil and in USA (11, 27, 28). However, in Brazil avoparcin is used in production farms. Frieden *et al.* suggested that the glycopeptide-resistance in enterococci was originated in hospitals, by inapropriate use, and then spread to the comunity (18).

In spite of the isolation of glycopeptides non-resistant enterococci (Table 3), important alterations in the cloacal microbiota of poultry fed with feed supplemented with avoparcin were detected. *E. faecium* was the major organism isolated in this group, in contrast with the control group, where another enterococci species, less pathogenic, prevailled. The use of avoparcin may be the cause, selecting the microbiota. All growth promoters have similar mecanisms of action, and select the most adapted organisms in the microbiota. It was reported that benzyl-penicillin in feed as growth promoter also selects *E. faecium* (19).

The bacteria selected by the antibiotic present in the feed is independent of the type of growth promoter used, but depends on the nature of the microbiota exposed to the antibiotic. Barrow showed that the use of avoparcin can reduce viable counts of

Table 1. Sources of enterococci isolated from faeces of chicken

Group	Number of birds	*Enterococcus sp*. isolated* n
Birds fed with feed with avoparcin:		
Test A (chicken living with 14-days-old)	33	38
Test B (chicken living with 21-days-old)	30	49
Test C (chicken with 35-days-old)	37	38
Total Test group (A, B and C)	100	125
Birds fed with feed without avoparcin:		
Control 1 (chicken living with 42-days-old)	30	43
Control 2 (chicken living with 25-days-old)	35	49
Total Control Group (1 and 2)	65	92
TOTAL	65	217

* cloacal swab

Table 2. *Enterococcus* species isolated from Control and Test chicken groups

	CONTROL GROUPS Feed without avoparcin N = 65 birds				TEST GROUPS Feed without avoparcin N = 100 birds				
	Control 1 N= 30 birds (42-days-old)	Control 2 N = 35 birds (25-days-old)	Total		A N = 33 birds (14-days-old)	B N = 30 birds (21-days-old)	C N = 37 birds (35-days-old)	Total	
Species*			n	%				n	%
E. casseliflavus	27	11	38	41.4	3	1	8	12	9.6
E. dispar	0	1	1	1.1	01	0	0	1	0.8
E. durans	1	2	3	3.2	2	5	0	7	5.6
E. faecalis	6	23	29	31.5	3	5	0	8	6.4
E. faecium	**6**	**5**	**11**	**11.9**	**21**	**27**	**18**	**66**	**52.8**
E. gallinarum	8	1	9	9.8	3	0	6	9	7.2
E. hirae	1	0	1	1.1	2	0	3	5	4.0
E. mundtii	0	0	0	0	2	0	3	5	4.0
E. raffinosus	0	0	0	0	1	11	0	12	9.6
Total	49	43	92	100	38	49	38	125	100

intestinal enterococci and Gram-negative anaerobic bacteria and an increase of *Salmonella typhimurium* and *E. coli*, confirming the antibiotic activity of avoparcin in the alimentary tract of chickens innoculated orally with a nalidixic acid-resistant *S. typhimurium* (8).

Differences in the proportion of each species, among enterococci in both control and test groups, were expected. Kaukas *et al.* (21) showed that the patterns of fecal microbiota change with poultry age and can be modified in birds treated with tylosin or ampicillin.

In view of the increasing nosocomial significance of *Enterococcus faecium* and *E. faecalis* and the alarming incidence of these oportunistic microorganisms in the Brazilian medical centers since 1997 (34), this study can contribute to elucidate some aspects of VRE epidemiology. Based in our results, the ecological impact of the use of avoparcin in farms can be better evaluated. A continuous surveillance program of glycopeptides resistant enterococci in animals and feed in Brazil is recommended, as well as the use of other antibiotics in the veterinary area.

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Table 3. Susceptibility to glycopeptides of enterococci isolated from faeces of chickens fed supplemented with ou without avoparcin

Group	Number of *Enterococcus sp* tested	Vancomycin* MIC Breakpoint ≥32µg/ml		Teicoplanin* MIC Breakpoint ≥32µg/ml		Avoparcin** MIC Breakpoint =8µg/ml	
	n	MIC_{50} µg/ml	MIC_{90} µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
Feed with avoparcin							
Test Group A	38	1	4	1	1	1	4
Test Group B	49	1	4	1	1	1	4
Test Group C	38	4	4	1	1	2	4
Feed withouth avoparcin							
Control Group 1	43	1	4	1	1	1	1
Control Group 2	49	2	2	1	1	4	4
TOTAL (N)	217						

* NCCLS (REF. 29, 30); ** Bager, DANMAP food (REF. 3); MIC, minimal inibitory concentration

MIC range found in the differents groups of enterococci:
Control Groups 1 and 2: vancomycin, 1- 4 µg/ml; teicoplanin, 1 µg/ml ; avoparcin, 1- 4 µg/ml
Test Groups A:vancomycin, 1- 8 µg/ml ; teicoplanin, 1- 4 µg/ml; avoparcin, 1- 4 µg/ml
Test Groups B:vancomycin, 1- 8 µg/ml; teicoplanin, 1 µg/ml; avoparcin, 1- 4 µg/ml
Test Groups C:vancomycin, 1- 4 µg/ml; teicoplanin, 1 µg/ml; avoparcin, 1- 4 µg/ml

RESUMO

Perfil de suscetibilidade a glicopeptídeos em enterococos isolados de frangos de um aviario de São Paulo, Brasil (1996-1997)

Para avaliar a resistência de enterococos de origem animal aos antibióticos glicopeptídeos foi projetado um estudo em aves comerciais que usavam rações suplementadas com avoparcina como promotor de crescimento. A suscetibilidade aos glicopeptídeos avoparcina, teicoplanina e vancomicina foi determinada em 217 enterococos isolados de fezes de frango colhidas através de swab cloacal (uma amostra/ave). Nos três grupos Teste foram usadas fezes de frangos em diferentes fases de crescimento, com 14, 21 e 35 dias de idade. As aves foram alimentadas com ração contendo avoparcina (10 mg/kg de ração) desde o primeiro dia de vida. Como controle foram usadas fezes de frangos do biotério da Faculdade de Medicina Veterinária da Universidade de São Paulo, onde nunca foram usados glicopeptídeos nas rações das aves ou no local. Nenhum enterococo resistente à vancomicina (ERV) foi isolado nas amostras examinadas, porém, foi detectado um aumento de *Enterococcus faecium* na microbiota fecal de frangos que utilizavam avoparcina na ração, independente da idade da ave.

Palavras-chave: glicopeptídeos, avoparcina, vancomicina, frangos, resistência a drogas, *Enterococcus*.

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