

**COMPARISON OF BsDA AND PREMI[®] TEST SENSITIVITY TO PENICILLIN
STANDARDS IN POULTRYMEAT AND AFTER ADMINISTRATION OF AMURIL
PLV. SOL.**

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ABSTRACT

For screening of antimicrobials in meat and meat products, a new microbiological test, Premi[®] test was developed. The sensitivity of the test was evaluated by using the meat juice saturated with penicillin's standards and the Premi[®] test sensitivity was compared with standard *Bacillus stearothermophilus* disc assay (BsDA). Both methods could detect the presence of penicillin's residues below the maximum residue limits (MRL), but detection limits of Premi[®] test were below the limits of BsDA. Premi[®] test is sensitive, reliable, easy-to-use and detects broad range of antibiotics.

Key words: Amuril plv. sol.; BsDA; penicillin residues; Premi[®] test

INTRODUCTION

The administration of antimicrobial drugs to food producing animals may lead to presence of residues in edible products. Growing concern among consumers and public health authorities on the presence of antibiotic residues in food products demand for a better control of both the animal production and human food products. As long as antibiotics are used on large scale in the intensive poultry farming, we have to be aware of risk for their presence in the food chain. Several concerns regarding the use of antibiotics are the antibiotic resistance, potential allergic reactions and technological problems of fermented meat products.

This situation leads to more strict regulations for occurrence of antibiotic residues in animal food products. Maximum residue limits (0.05 mg.kg^{-1} for penicillin, ampicillin, amoxicillin, 0.3 mg.kg^{-1} for oxacillin, cloxacillin) have been established to prevent too high concentration of antibiotics. There is a consumer's expectation that the food products do not contain over limited concentrations of antibiotics.

Therefore the availability of simply and reliable screening systems for the detection of antibiotics is an essential tool in assuring the safety of food products. Recently a new broad spectrum screening test for the detection of antimicrobial residues in meat, Premi[®] test, has been developed (6, 7).

In our experiment sensitivity of Premi[®] test on different concentrations of 5 penicillins and Amuril p/v. sol. were tested. The results were compared with standard microbiological BsDA method.

MATERIAL AND METHODS

Penicillins including penicillin, ampicillin, amoxicillin, oxacillin, and cloxacillin were purchased from Sigma (St. Louis, MO, USA). Amuril plv. sol. (amoxicillinum 10 g in 100 g of powder) was obtained from Lek (Slovenia). *Bacillus stearothermophilus* var. *calidolactis* disc assay (BsDA) consists of agar and suspension was obtained from Merck (Germany). Premi[®] test was taken from DSM (Netherlands) and Thermoblock (Biotech, Slovakia) was used as a block heater for Premitest ampoule incubation.

Separate stock solutions of 25 mg per 25 ml of penicillin, ampicillin, amoxicillin, oxacillin, and cloxacillin were prepared by dissolving in deionised water. Working standard solutions (WSS) and model meat juice samples of tested antibiotics were then prepared as described in Table 1. Meat juice samples for the estimation of the detection limits of tests were extracted by using of garlic press from the antibiotic free poultry meat and the meat juice was used as a negative control. WSS were stored in a cool place (2–4 °C) protected from light.

Bacillus stearothermophilus var. *calidolactis* disc assay (BsDA) utilises a spore suspension of *Bacillus stearothermophilus* var. *calidolactis* inoculated into indicator agar (Merck, Germany). The paper disc soaked with a meat juice standard sample was placed on the surface of the agar. The plates were incubated at 64 °C for 3 hours and were observed for production of clear zones of inhibition around the sample discs. Inhibition zones are indicated in mm, the positive zone is read at the size above 2 mm.

Premi[®] test (DSM, The Netherlands) ampoule method for antibiotic residue detection utilises a culture medium containing *Bacillus stearothermophilus* var. *calidolactis*. Premi[®] test combines the principle of agar diffusion test with colour change of the indicator in consequence of the active metabolism of the tested microorganism. 100 µl of the meat juice

was placed onto the agar in the ampoule and incubated 20 minutes at the room temperature for a prediffusion. The meat juice was not flushed away from the ampoule. The ampoules were incubated for 3 hours at 64 ± 1 °C and change of the colour was evaluated.

To determine the sensitivity of the screening tests each concentration was replicated at least 4–5 times.

In our experiment 30 laying hens (ISA brown 220) were used and the weight average was 2.3 kg. Amuril (dosage 0.5 g / laying hen / day) was administered with sonda into the oesophagus 4 times in 24 hour intervals. Zero control hens were slaughtered before beginning of administration and slaughtering continued every day (4 hens daily) until the time when the all results were negative.

Meat juice samples were extracted by using of garlic press from the breast muscles of the laying hens and the meat juice from antibiotic free poultry was used as a negative control.

Data were analysed by Win Episcope 2.0 using test agreement and kappa value for various assay systems comparison.

RESULTS AND DISCUSSION

The results, in which a different concentration of penicillin G, ampicillin, amoxicillin, oxacillin, and cloxacillin were added to poultry meat juice are summarised in Table 2. The model meat juice samples were analysed by using of BsDA, and Premi[®] test.

BsDA produced clear zones of inhibition and the highest sensitive was recorded for penicillin G with minimum detection limit 0.005 mg.kg^{-1} . In case of ampicillin and amoxicillin detection limits were 0.010, respectively 0.025 mg.kg^{-1} and the lowest sensitivity was obtained from cloxacillin and oxacillin estimation (0.040 mg.kg^{-1}).

The Premi[®] test could detect the presence of penicillin G more sensitive than BsDA ($\kappa > 0.6$) with detection limit 0.004 mg.kg^{-1} and similar results were recorded also for ampicillin and amoxicillin (0.005 mg.kg^{-1}) and for cloxacillin and oxacillin (0.030 mg.kg^{-1}).

Both methods, detected presence of penicillins residues in poultry meat juice below the permitted maximum residue limits established in Food Code (0.050 mg.kg^{-1}) (3).

Bacillus stearothermophilus disc assay (BsDA) is a standard test recommended by Governmental veterinary and food administration (2) for antibiotic residues detection in food and foodstuff of animal origin. As an alternative of BsDA, Premi[®] test was prepared. This easy-to-use test should guarantee that producers could prevent the risk of penalties because of positive meat samples. The principle of the test is similar to Delvotest SP that is routinely used for antibiotic residue detection in milk in milk industry. The Premi[®] test should be applied in the same way in meat processing facilities.

R e y b r o e c k (4) tested concentrations of residues of different substances in the different naturally contaminated meat samples. Each meat sample was originated from a different chicken and Premitest fulfilled conditions of European legislation. The Premi[®] test was used by R e y b r o e c k (5) for spiked meat juice samples estimation and the results of Premi[®] test sensitivity for ampicillin (0.005 mg.kg^{-1}), amoxicillin (0.005 mg.kg^{-1}), oxacillin (0.025 mg.kg^{-1}), and cloxacillin (0.040 mg/kg) were comparable with our results (0.005 , 0.005 , 0.030 , 0.030 mg.kg^{-1}). A r t s and W i t k a m p (1) performed improvement of screening of antimicrobial drug residues. Minimum detection levels were $0.0025 \text{ mg.kg}^{-1}$ for penicillin G, and 0.005 mg.kg^{-1} for ampicillin and amoxicillin.

Premi[®] test integrated strategy of antimicrobial compound detection at or below the MRL in a broad spectrum of food products including meat. Proper use of Premi[®] test will contribute to less positive animals, safer products and better consumer protection.

In our animal study the presence of amoxicillin residues after experimental administration in laying hens was evaluated.

With the BsDA amoxicillin residues were detected in meat juice from the breast muscles, breast and thigh muscles, kidney, heart, and liver. Comparison of zones of inhibition from meat juice samples and muscles placed direct on to agar medium showed that meat juice produced significantly ($p < 0.005$) larger zones than muscle samples.

Premi[®] test was used for residue determination in meat juice from breast muscles. Amoxicillin that could be detected with the BsDA could always be detected with Premi[®] test. The last positive results of BsDA were recorded 24 hours after finishing of administration and Premi[®] test 48 hours. It means, in comparison with BsDA using the Premi[®] test in this experiment amoxicillin residue could be detected in poultry muscles for a 24 hours longer period after treatment.

BsDA analysis of heart and kidney showed that all zones of inhibition were below 2 mm and must be considered as a negative. The liver produced positive results only at the time of Amuril plv. sol. administration.

All results of BsDA and Premi[®] test, on the 3rd day after finishing of drug administration, were negative. On the basis of these results, both methods did not exceed withdrawal period recommended by producer (2 days).

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Table 1. Concentration ranges of tested antibiotics in model meat juice samples

Antibiotic	Concentration ranges (mg.kg⁻¹)
Penicillin G	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Ampicillin	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Amoxicillin	0.001; 0.002; 0.003; 0.004; 0.005; 0.010; 0.025; 0.050; 0.100
Oxacillin	0.010; 0.020; 0.030; 0.040; 0.050; 0.100; 0.200
Cloxacillin	0.010; 0.020; 0.030; 0.040; 0.050; 0.100; 0.200

Table 2. Results of BsDA and Premi[®] test means in poultry meat juice sample models

Antibiotics	Tests	Concentration ranges (mg.kg⁻¹)							
		0.003	0.004	0.005	0.010	0.025	0.050	0.100	0.200
Penicillin G	BsDA*	0.5	0.5	2.0	3.0	3.5	5.0	7.0	
	Premi [®] test	--	+	+	+	+	+	+	
Ampicillin	BsDA*	0	0	1.0	2.0	3.0	4.5	6.0	
	Premi [®] test	--	--	+	+	+	+	+	
Amoxicillin	BsDA*	0	0	1.0	1.5	2.0	3.5	5.0	
	Premi [®] test	--	--	+	+	+	+	+	
Cloxacillin	BsDA*	0	0	1.0	2.5	3.5	5.0	7.0	
	Premi [®] test	--	--	+	+	+	+	+	
Oxacillin	BsDA*	0	0	1.0	2.0	3.0	4.5	6.0	
	Premi [®] test	--	--	+	+	+	+	+	

* - zones of inhibition (mm)

Table 3. Comparison of mean values of BsDA and Premi[®] test after experimental administration of Amuril plv. sol. in laying hens

Time (hours)	BsDA					Premi [®] test (breast muscles)
	Breast**	Thigh	Kidney	Heart	Liver	
0*	0 / 0	0	0	0	0	--
24*	3.3 / 6.7	1.7	1.7	0	2.3	+
48*	4.7 / 7.7	1.7	1.3	1.3	2.0	+
72*	1.8 / 4.7	1.7	1.7	0.7	2.0	+
96	2.3 / 4.0	1.7	0.3	0	1.7	+
120	0.7 / 1.3	0.3	0.3	0	1.0	+
144	0.3 / 0.7	0.3	0.7	0.3	1.3	±
168	0 / 0	0	0	0	0	--

* - Amuril plv. sol. administration

** - Zones of inhibition from breast muscles / breast muscles juice