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Development of New Lower-Cost Alternative Methods for Screening of Antibiotic Residues in Meat.

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ABSTRACT

Our study targets the development of microbiological methods alternative, effective and economic, for the screening of antibiotic residues in red and white meat. Two methods are developed and compared with conventional methods: a one plate-method and a tube-method. The results show that the one plate-method is effective with the Mueller-Hinton medium inoculated with *Micrococcus luteus*. This method presents the same results as the Prémi®test. It is promising as a screening alternative method: it is sensitive and does not detect false positives. The tube-method presents significant results for the Mueller-Hinton medium supplemented with Bromothymol Blue at 0.01 g/l, containing NaCl at a concentration of 50g/l and inoculated with *Micrococcus luteus* at 144,109 CFU/ml. The optimal test volume of meat juice is 100µl. The incubation time recorded under these conditions is 2h-2h 30min. In conclusion, alternative screening methods, sensitive and adapted to our economic means have been developed. A validation is recommended to prove its application.

Keywords: Four plate Method, STAR Method, Screening Methods, Prémi®test, one plate Method, The tube method, Micrococcus luteus.

1 Introduction

Antibiotic residues analysis in meat poses an economic problem for certain countries, hence the need for simple, sensitive and inexpensive screening methods. Our study targets the development of microbiological methods alternative, effective and economic, for the screening of antibiotic residues in red and white meat.

2 Experimental

The alternative one plate-method was developed on 61 samples (18 positive and 43 negative) using two media (Soy Agar and Mueller/ Hinton) and three test strains (*Micrococcus luteus, Bacillus stearothermophilus and Staphylococcus aureus*).The alternative tube-method was developed on 26 samples (12 positive and 14 negative). The optimized parameters are the nature and concentration of the colored indicator (bromothymol blue, phenol red and bromocresol purple), the concentration of the inhibitor agent (NaCl), the load of the medium in *Micrococcus luteus* strain and the volume of the sample (Meat Juice). Samples are pre-analyzed by conventional methods: the *Prémi®test for screening* [01] and the reference method for confirmation: the four-plate test [02] supplemented with one plate of STAR method (for quinolone screening) [03]. In parallel, three negative control samples were prepared from chicken farms without antibiotics.

3 Results and discussion

The results show that the one plate-method is effective with the Mueller-Hinton medium inoculated with Micrococcus luteus. The analysis of the 61 samples (+ three negative controls) under these conditions reveals that the method test presents the same results as the Prémi®test. It is promising as a screening alternative method: it is sensitive and does not detect false positives. Although its long incubation period (24 hours), it has a significant economic competitiveness.The tube-method presents significant results for the Mueller-Hinton medium supplemented with Bromothymol Blue at 0.01 g/l, containing NaCl at a

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concentration of 50g/l and inoculated with Micrococcus luteus at 144,109 CFU/ml. The optimal test volume of meat juice is 100µl. The incubation time recorded under these conditions is 2h-2h 30min.

4 Conclusions

In conclusion, alternative screening methods, sensitive and adapted to our economic means have been developed. Validation with a real economic estimate (price/analyzed sample) is desirable to prove its application.

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