Microbial Inhibition Test

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Antimicrobial detection tests are conventional screening tools used in slaughterhouses to prevent the entry of antimicrobial residues into the food chain. The occasional appearance of antibiotic or bacteriostatic residues is a problem of major worldwide concern, as such residues can lead not only to toxicity for humans, but also to the emergence of antimicrobial resistance (AMR). In particular, antibacterial residues that contaminate meat can cause allergic reactions, can lead to dysbiosis of the gastrointestinal flora and can enhance dissemination of AMR, not only in the environment but also inside the gut, leading to antibacterially resistant communities in our intestinal flora.

Keywords: antibiotics ; sulfonamides ; antimicrobials ; blood ; in vivo ; biological test

1. Overview

Even though antibiotics are necessary in livestock production, they can be harmful not only due to their toxicity, but also in view of their contribution to the emergence of antimicrobial resistance. Screening tests based on microbial growth inhibition appeared to be useful tools to prevent their entry into the food chain. They have nevertheless been traditionally carried out post mortem, leading to great economical loss and harm to the environment in case a positive sample is found. Hence, the objective was to evaluate the use of a screening test as an ante mortem alternative for the detection of antibiotic residues in meat: thus, Explorer®-Blood test was optimized and validated. After adapting the procedure for matrix preparation, the assay parameters were assessed from 344 antibiotic-free blood serum samples. Limits of Detection (LoDs) were defined by spiking blood serum with several of the most common antimicrobials used in veterinary practice. LoDs were similar to those obtained for meat and were at or below the maximum residue limits set by EU legislation for muscle. Analyses of in vivo injected samples, previously characterized by LC-MS/MS, demonstrated the method's accuracy and proved that Explorer®-Blood can be considered a suitable alternative to conventional post mortem screening methods.

2. Antimicrobial Detection Tests

Antimicrobial detection tests are conventional screening tools used in slaughterhouses to prevent the entry of antimicrobial residues into the food chain. The occasional appearance of antibiotic or bacteriostatic residues is a problem of major worldwide concern, as such residues can lead not only to toxicity for humans, but also to the emergence of antimicrobial resistance (AMR) [1][2]. In particular, antibacterial residues that contaminate meat can cause allergic reactions, can lead to dysbiosis of the gastrointestinal flora and can enhance dissemination of AMR, not only in the environment but also inside the gut, leading to antibacterially resistant communities in our intestinal flora [3][4][5][6]. Even though the development of AMR is the most critical issue as it is responsible for the loss of effectiveness of antimicrobials against common infectious diseases, less studied aspects, such as the unknown toxicity of their derivates after cooking meat [7] or the presence of residues of oxidative antimicrobials that disrupt the endogenous antioxidant system [8], should likewise be taken into consideration.

In order to address this problem and create a safe environment for consumers, the European Union (EU) has established an extensive legislative framework by restricting the authorization of medicinal products allowed for veterinary use [9], by determining maximum residue limits (MRLs) [10], by setting safe withdrawal periods [11], by monitoring plans [12] and by establishing a series of analytical methods for the testing of official samples [13].

A wide range of methods for the analysis of antibiotic residues in meat are currently available. In Europe, methods for official control are classified as screening and confirmatory methods. The most common surveillance programs for antibiotic residue control start by screening a large number of samples in a short time with easy, inexpensive methods [14]. Screening methods must detect a broad spectrum of antimicrobials at the regulatory levels; ideally, no more than 5% false compliant results should be accepted. Moreover, presumptive non-compliant results must be confirmed with a suitable validated method [13].

Among recommended screening methods, several commercialized tests for the detection of antibiotics in food matrices based on growth inhibition of microorganisms are available. Many of them inhibit the growth of microorganisms such as *Geobacillus stearothermophilus* when antibacterial residues are present in a sample [14]. Further simplification and automatization of this kind of assays has been proposed in recent years. An example of such methods is the Explorer® test, initially validated for the analysis of muscle [15] and eggs [16], and currently coupled to e-Reader® device, that performs incubation, reading and interpretation of the assay result. Explorer® tests are presented in a tube in which a specific detection media is spread with the target microorganism. A growth indicator is additionally included in the medium, usually based on change of pH or redox potential. When the target microorganism grows (thereby indicating that the sample contains no antibiotics, or that their residues lie below the method's limit of detection), the test medium colour changes from blue/purple to yellow/orange (sample is considered as negative). However, if the sample contains antimicrobials, metabolism of the bacteria is stopped or slowed down and no colour change or only a partial colour change is visible (sample is considered as positive).

Surveillance of antibiotic residues in foods of animal origin is carried out post mortem. If a positive sample is found to have amounts of antibacterial residues that are over the limits set by legislation (MRLs), carcasses must be confiscated and destroyed. Such an event has devastating repercussions for the farmer, who has to face substantial financial loss caused by severe fines, as well as the investment lost in breeding animals of no value. In addition, one must consider the harmful environmental impact of breeding livestock that ends up as waste, associated with the misuse of input resources and the release of contaminating output such as gas emissions, manure/slurry, residual water and the challenge of destroying the carcasses [17].

The analysis of antimicrobial residues in animals prior to slaughter has thus been attracting increased attention in recent years [18][19]. Several issues need to be solved, however, before implementing a methodology for in vivo testing for antibiotics. First of all, the selection of the most suitable matrix (tissue or biological fluid) is an essential requirement. A matrix for antibiotic detection should be easy to collect and should be representative of the level of antimicrobial substances found in edible tissues (e.g., muscle). To ascertain this, several studies comparing the concentration of antibacterial compounds in body tissues and fluids have been published [20][21][22][23][24]. A most recent study carried out on pigs demonstrated that blood serum was the most suitable matrix for laying the bases of a new in vivo antimicrobial detection test [25], as the concentration of antimicrobial molecules in blood serum (**Figure 1a**) showed an acceptable equivalence with that found in muscle (**Figure 1b**). Moreover, the collection of blood from living animals is a simple practice that is commonly carried out in farms.

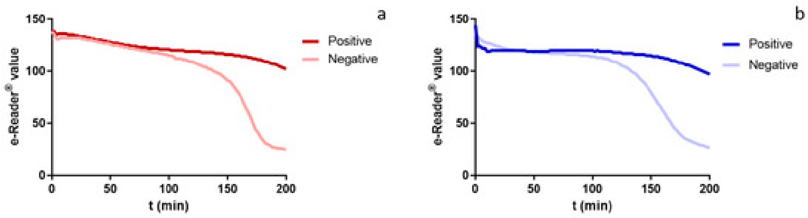

Figure 1. Colour change kinetics registered by e-Reader® for positive (dark line) and negative (light line) blood serum samples analysed with Explorer®-Blood test (**a**) and muscle samples analysed with Explorer® (**b**).

The implementation of systems for ante mortem screening of antibiotics in livestock by testing blood serum samples could help to overcome the aforementioned limitations of post mortem analysis. In order to be put into practice as a routine plan, the method needs to be rapid and affordable, allowing for decision-making within a few hours. However, no simple and automatic method has yet been adapted for the screening of antimicrobials in blood.

3. Conclusions

A post mortem method for the screening of antimicrobials in meat has been adapted as an ante mortem tool for the analysis of antibiotics and sulfonamides in blood serum. The new method was validated and compared with results obtained in meat, thereby proving that the Explorer®-Blood test coupled to e-Reader® is a suitable method for ante mortem implementation at farm or slaughterhouse level. On the one hand, blood serum proved to be the ideal matrix, as it is easy to obtain and prepare for analysis and its antimicrobial concentrations showed a satisfactory equivalence with muscle in the case of most antimicrobials [25]. On the other hand, this method's adaptation only required a small number of modifications, thereby maintaining its ease of analysis. The new Explorer®-Blood test can be carried out by non-qualified personnel, at any location, and within a brief time interval. Hence, it can be regarded as a pioneering tool for the analysis of antibacterial residues in living animals, thereby ensuring the absence of such residues in meat while preserving the agricultural production economy as well as consumer health.

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