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ORIGINAL ARTICLE

VERIFICATION OF THE PRESENCE OF ANTIBIOTIC RESIDUE IN SHEEP MEAT

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Antibiotics are substances with a direct action on bacteria that are used for treatment or prevention of infectious diseases. Their usage in food-producing animals may result in deposition of their residues in animal products. The sheep sector is considered an overall lower user of antibiotics, but there is still a need to monitor residues in sheep meat also. The aim of this study was to verify whether antibiotic residues are present in samples of sheep meat on the market from various local suppliers in Ireland, and if so, to tentatively identify them. Our study was carried out using two microbial inhibition tests, the tube Explorer 2.0 test with photometrical reading of the results and the multi-plate Screening Test for Antibiotic Residue (STAR), for the antibiotic group identification in the samples tested positive. Out of the 15 random samples tested, 2 samples yielded a positive result using the Explorer 2.0 test. Using the STAR, both positive samples were considered suspicious for the presence of beta-lactam antibiotics. Another fresh sample from each supplier that tested positive already had a negative result. Reliable monitoring of meat from food-producing animals in accordance with European Union law contributes to the protection of public health and strengthens consumer confidence.

Key words: antibiotic residues; screening; sheep meat

INTRODUCTION

"Antibiotic" means any substance with a direct action on bacteria that is used for treatment or prevention of infections or infectious diseases [1]. Antibiotics bring both benefits and adverse effects, but they need to be used in a responsible manner to prolong their efficacy. Benefits of using antibiotics in food-producing animals include health and welfare purposes of the animal, carcass quality, growth and production efficiency, economics, protection of the environment, decreased morbidity and mortality, and human health [2]. Adverse effects of residues on human health include development of drug resistance, carcinogenicity, mutagenicity, hypersensitivity reaction, bone marrow de-

pression and toxicity, disruption of normal intestinal flora, immunopathological effects, autoimmunity, and teratogenic effects [3, 4].

Strict EU legislations exist around the levels of residues of pharmacologically active substances, including antibiotics, known as maximum residue limits (MRLs), which must be officially controlled in food-producing animals and foods of animal origin. MRL means the maximum concentration of a residue of a pharmacologically active substance, which may be permitted in food of animal origin [5]. MRLs for pharmacologically active substances authorised as veterinary medicinal products are set out in the Commission Regulation (EU) 37/2010 [6]. Regulation (EU) 2017/625 [7] and its delegated and implementing acts lay down specific rules for the performance of official controls in relation to substances whose use may result in residues in food and feed. Commission Delegated Regulation (EU) 2022/1644 [8] lays down rules for the performance of official controls in regard to the range of samples and the stage of production, processing and distribution, in which the samples are to be taken regarding the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof. The uniform practical arrangements for the performance of official controls as regards the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof, on specific content of multi-annual national control plans and specific arrangements for their preparation, are set in Commission Implementing Regulation (EU) 2022/1646 [9]. The rules on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling, are laid down in the Commission Implementing Regulation (EU) 2021/808 [10].

Analytical methods can be classified into five different cases based on the type of analysis (screening, confirmation) and the mode of analyte determination (qualitative, semi-quantitative, quantitative) [11]. “Screening method” means a method that is used for screening of a substance or class of substances at the level of interest (MRL). “Confirmatory method” means a method that provides full or complementary information enabling the substance to be unequivocally identified and, if necessary, quantified at or below the maximum residue level for authorised substances [10].

The current residue control strategy is based on two sequential steps: screening and confirmation. Screening techniques can generate qualitative (positive or negative), semiquantitative (high, medium/low), or quantitative results [12]. They are based on different detection principles (e.g., microbiological, immunological, physico-chemical) and must be able to screen for potential non-compliant results [13, 14]. A sample categorized as potentially non-compliant (“Screen Positive”) is subsequently analysed with a confirmatory test [15].

There are no restrictions for screening methods regarding the suitability of specific approaches or detection principles [15]. Microbial inhibition tests (MIT) are commonly used as screening tests for antibiotic residues, as they are relatively cheap, easy to perform, and have a broad spectrum of sensitivity. These screening tests are based on the principle of bacterial growth inhibition produced by concentrations of antimicrobial residues above the limit of detection of the test. There are two main groups of broad-spectrum MITs: tube and multi-plate tests [16, 17, 12, 18, 19]. Multi-plate tests provide more information than tube tests but in turn are more laborious and therefore more suited to official laboratories, whereas a slaughterhouse required to analyse a large number may find tube tests more suitable [20].

Tube tests have the added advantage of being ready to use, and results are available comparatively faster than multi-plate tests. Generally, tube tests consist of a medium (agar base and nutrients) seeded with the susceptible bacteria and a growth indicator (pH or redox based). The interpretation of the result is based on a colour change of the medium. Generally, a colour change indicates antibiotics are not present, and a lack thereof would imply a non-compliant sample [19, 21]. Plate or multi-plate test systems are based on a combination of different test organisms that are sensitive to a group or groups of antibiotics. The interpretation of the result is based on the production of an inhibition zone. The diameter of the inhibition zone theoretically correlates with the concentration of the antimicrobial compound in the sample [18].

Many MITs have been developed or commercialized for the screening of antibiotic residues in the meat

of food-producing animals. Among the most widely used ready-to-use tube tests for the screening of antibiotic residues in meat and offal of food-producing animals are the Premi®Test produced by R-Biopharm in Germany and the Explorer 2.0 test developed by Zeulab in Spain. Both tests rely on agar diffusion of the antibiotic residues, which is expressed by the absence of colour change from purple to yellow. The advantage of the Explorer 2.0 test over the Premi®Test is the interpretation of results by measuring colour change with the e-Reader. The e-Reader uses internal software to integrate time and colour parameters to determine the endpoint of the assay, stopping automatically and interpreting qualitative results. Performance of the Explorer 2.0 combined with an automated e-Reader device for the detection of antimicrobial residues in muscle was validated in line with MRL according to European Commission Decision 2002/657/EC [22] by Mata et al. (2014) [20]. Premi®Test was validated for the detection of antimicrobial residues in animal muscle tissues according to AFNOR rules (French Association for Normalisation) by Gaudin et al. (2008) [23].

The plate methods developed so far are using one to seven plates, different pH and media combinations, and one or different bacterial strains to improve the capability detecting different antibiotic groups [24]. Samples are considered positive if they gave the inhibition zone around the sample or the punch hole in the diameter set by the method. The most widely used plate method is the five-plate test known as the Screening Test for Antibiotic Residues (STAR), which was developed by the AFSSA Community Reference Laboratory in Fougeres for screening antimicrobial residues in muscle [25].

As antibiotic residues continue to be a public health concern, the aim of our study was to verify the presence of antibiotic residues in sheep meat originating from supermarkets and butchers in Ireland using the Explorer 2.0 test in conjunction with the e-Reader, followed by preliminary identification using the STAR method.

MATERIALS AND METHODS

Sheep meat samples

A total of 15 sheep meat samples for this study were collected from butchers and supermarkets in Ireland over several months. All fifteen samples were from a shoulder cut of the carcass. Samples were stored in a freezer at -18 °C before the analysis.

Explorer 2.0 test coupled to e-Reader

The first laboratory test that was carried out was a qualitative test, Explorer 2.0, developed by Zeulab S.L. (Zaragoza, Spain) with e-Reader (the equipment that incubates, stops the assay, and interprets the results). The principle of the Explorer 2.0 test is based on the inhibition of microbial growth. The kit is supplied in a single-tube format. Each tube contains agar medium spread with *Geobacillus thermophile* spores and a pH indicator. When the test is incubated at 65 °C, spores germinate, and cells grow, producing acid and changing the agar pH. Variations of pH will produce changes of the agar colour from purple to yellow. When samples contain inhibitors at higher concentrations than the limit of detection, the bacteria will not grow, and no colour change will be observed.

Sample preparation and assay procedure for the Explorer 2.0 test

3 g ± 0.5 g of lean meat muscle without adipose or connective tissue was weighed in a screw top tube. The tube was closed slightly but not fully sealed. The tube was put in a beaker of water. The beaker was then put in a microwave on the defrost setting for 3-4 minutes, until the meat was fully cooked. The meat was then removed, and the meat juice was used for analysis. A lyophilized negative control sample provided by Zeulab S.L. (ZE/EX/CN5) was used to determine the optimal incubation of the assay in each run.

100 µl of the negative control sample and 100 µl of each sample were pipetted onto the surface of the agar medium in the test tube. The tubes were then incubated at room temperature for 20 minutes. After 20 minutes, the tubes were turned upside down to remove the remaining sample. The tubes were washed with distilled water. The water was removed by placing the tube upside down and dried on absorbent paper. The tubes were sealed with adhesive foil and placed into the e-Reader for incubation at the temperature of 65 ºC. The procedure was terminated automatically, and results were read and displayed on the screen. The device detects a colour change of the negative control by photometric reading and automatically terminates the incubation time of the samples, with a positive sample expressed as a value ≥56 and a negative sample as a value <56.

STAR method

The method used for sample analysis is adopted from the STAR protocol developed by the Community Reference Laboratory in Fougeres, described by Gaudin et al. (2010) [25].

Test organisms and agar media used in the STAR protocol

Five culture media were used: 1) *Bacillus subtilis* BGA 5 x 10^4 spores/mL (Merck 10649, Darmstadt, Germany), Antibiotic medium 11 (Difco 259310, Detroit, USA), pH 8.0; 2) *Kocuria rhizophila* ATCC 9341 5 x 10^4 germs/mL (Czech Collection of Microorganisms, Brno, Czech Republic), Merck 10664 medium (Darmstadt, Germany), pH 8.0; 3) *Bacillus cereus* ATCC 11778 3 x 10^4 germs/mL (Czech Collection of Microorganisms, Brno, Czech Republic), Merck 10663 medium (Darmstadt, Germany), pH 6.0; 4) *Escherichia coli* ATCC 11303 10^5 germs/mL (Czech Collection of Microorganisms Brno, Czech Republic); Merck 10664 medium (Darmstadt, Germany), pH 8.0; 5) *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 5 x 10^5 spores/mL (Merck 1.11499, Darmstadt, Germany); Diagnostic Sensitive Test agar (Oxoid CM 261, Basingstoke, United Kingdom), pH 7.4 supplemented with trimethoprim (Fluka 92131, Buchs, Switzerland) 0.005 µg/mL. Culture media were prepared according to the manufacturer's instruction.

Sample preparation and assay procedure for the STAR method

5 ml of inoculated medium was added to each Petri dish. A cylindrical plug of 8 mm in diameter and 2 cm in length was cut in frozen muscle using a cork borer. Both ends of the cylindrical core were discarded, and 2 mm thick slices of muscle were cut by a sterile lancet. Two tissue discs were placed on one plate directly across from each other. The plates are then incubated as follows: *Bacillus subtilis* BGA for 18 hours at 30 °C, *Escherichia coli* ATCC 11303 for 18 hours at 30 °C, *Kocuria rhizophila* ATCC 9341 for 24 hours at 37 °C, *Bacillus cereus* ATCC 11778 for 18 hours at 37 °C, and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 for 15–16 hours at 55 °C. A sample is positive when the inhibition zone around the sample is equal to or more than 2 mm on *Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303, *Kocuria rhizophila* ATCC 9341, and *Bacillus cereus* ATCC 11778 test plates, and equal to or more than 4 mm on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates. The diameters of the inhibition zones were measured by an electronic caliper (Mitutoyo, Kawasaki, Japan). The diameter of the inhibition zones was expressed as the mean ± standard deviation (SD) of six measures.

Two paper discs (9 mm, Whatman No. 1, Maidstone, United Kingdom) soaked with 30 µg/L of control standard solutions of reference antibiotics were placed on the surface of the agar medium to verify the sensitivity of the plates. The following control solutions were used: sulfamethazine (S 5637, Sigma, St. Louis, MO, USA) at 1000 µg/L on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 plate, streptomycin (S 6501, Sigma, St. Louis, MO, USA) at 2000 µg/L on *Bacillus subtilis* BGA plate, tylosin (T 6134, Sigma St. Louis, MO, USA) at 1000 µg/L on *Kocuria rhizophila* ATCC 9341 plate, chlortetracycline (C4881, Sigma, St. Louis, MO, USA) at 200 µg/L on *Bacillus cereus* ATCC 11778 plate, and ciprofloxacin (17850, Fluka Chemie GmbH, Buchs, Switzerland) at 100 µg/L on *Escherichia coli* ATCC 11303 plate. The diameters of the inhibition zones around the positive control paper discs should present the inhibition zones as follows: streptomycin 4.5 ± 1.5 mm, tylosin 5.5 ± 1.5 mm, chlortetracycline 6.0 ± 1.5 mm, ciprofloxacin 5.5 ± 1.5 mm, and sulphamethazine 5.0 ± 1.5 mm.

RESULTS

The results of the Explorer 2.0 test were obtained from the e-Reader when the assay reached the endpoint that was electronically determined by the e-Reader itself. The results were displayed on the screen, and the colour indicated the sample as positive (red) or negative (green). The test results of the Explorer 2.0 test are shown in Table 1, with sample 7 and sample 11 being positive. As can be seen from Table 1, the e-Reader coupled to the Explorer 2.0 test determined the test endpoint at a cut-off of 56 and generated the results as negative (<56) or positive (>56). The cut-off of a screening test is the response or signal, which indicates that a sample contains an analyte at or above the target concentration, thereby ensuring discrimination between positive and negative samples [15]. The test results from the e-Reader are shown in Figures 1, 2, and 3.

Table 1. Explorer 2.0 test results

Sample No.	Source	Explorer 2.0	Cut-off value
1	Supermarket	Negative	<56
2	Supermarket	Negative	<56
3	Butcher	Negative	<56
4	Supermarket	Negative	<56
5	Supermarket	Negative	<56
6	Butcher	Negative	<56
7	Butcher	**Positive**	**57**
8	Supermarket	Negative	<56
9	Supermarket	Negative	<56
10	Supermarket	Negative	<56
11	Butcher	**Positive**	**95**
12	Butcher	Negative	<56
13	Butcher	Negative	<56
14	Supermarket	Negative	<56
15	Butcher	Negative	<56

Fig. 1. Results of Explorer 2.0 with e-Reader for samples 1-5

When the Explorer 2.0 test was repeated with new sheep meat samples from both positive suppliers, the results for both samples were negative, as shown in Table 2. The test results generated by the e-Reader as negative for both new samples (green, cut-off <56) are shown in Figure 4.

Fig. 2. Results of Explorer 2.0 coupled to e-Reader for samples 6-10

Fig. 3. Results of Explorer 2.0 coupled to e-Reader for samples 11-15

Table 2. Explorer 2.0 test results for additional samples from positive suppliers

Sample No.	Source	Explorer 2.0	Cut-off value
7a	Butcher	Negative	<56
11a	Butcher	Negative	<56

Fig. 4. Results of Explorer 2.0 coupled to e-Reader for additional samples from positive suppliers

As sample 7 and sample 11 had a positive result from the Explorer 2.0 test coupled to e-Reader, the STAR method was used to confirm the positive results and to preliminarily identify antibiotics in these samples. Both positive food samples showed the formation of inhibition zones on the plates seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 and *Kocuria rhizophila* ATCC 9341. The mean diameter of the inhibition zone on the plate seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 was 2.86 ± 0.29 mm for sample 7 and 4.89 ± 0.42 mm for sample 11. The mean diameter of the inhibition zone on the plate seeded with test organism *Kocuria rhizophila* ATCC 9341 was 2.76 ± 0.35 mm for sample 7 and 2.91 ± 0.16 mm for sample 11. According to the width of the inhibition zones set for the positive results on the plate seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149, sample 7 was considered negative and sample 11 positive for antibiotic residues. According to its sensitivity to a group of antibiotics, sample 11 is suspected of the presence of beta-lactams or sulphonamides. According to the width of the inhibition zones set for the positive results on the plate seeded with test organism *Kocuria rhizophila* ATCC 9341, both samples were considered positive for antibiotic residues. Based on its sensitivity to a group of antibiotics, the samples are suspected of the presence of macrolides and beta-lactams. No inhibition zones were observed on the plates seeded with other test organisms of the STAR method. The mean diameter of the inhibition zones around the samples detected positive by the Explorer 2.0 test coupled with the e-Reader is presented in Tables 3 and 4.

Table 3. Mean diameter of the inhibition zones around the samples detected on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates

Sample No.	Source	Diameter, mm ± SD	Result
7	Butcher	2.86 ± 0.29	Negative
11	Butcher	4.89 ± 0.42	Positive

SD – standard deviation. All data are the mean ± SD of six measurements.

Table 4. Mean diameter of the inhibition zones around the samples on *Kocuria rhizophila ATCC 9341* test plates

Sample No.	Source	Diameter, mm ± SD	Result
7	Butcher	2.76 ± 0.35	Positive
11	Butcher	2.91 ± 0.16	Positive

SD – standard deviation. All data are the mean ± SD of six measurements.

The susceptibility of test organisms to control antibiotics determined by the STAR method was confirmed before analysis. This was done by creating inhibition zones around positive control paper discs with diameters corresponding to the sizes of the inhibition zones set for control antibiotics by the STAR method.

DISCUSSION

The direct risk to human health posed by antibiotic residues in food has been well documented. Direct risks include hypersensitivity reactions, which may range from a cutaneous reaction to acute anaphylaxis, liver injury, and disruption of the human intestinal microbiome in cases of short-term exposure to antibiotic residues [16, 26]. Long-term exposure can result in carcinogenicity, teratogenicity, and mutagenicity. Coupled with these concerning direct health risks, the indirect threat from antibiotic residues in food is that of antimicrobial resistance. There is no doubt that the use of antibiotics in livestock leads to antibiotic resistance in human medicine. However, antibiotics are important in modern farming practices for maintaining animal health and animal welfare and food production standards [16].

Due to the many negative implications posed to public health by antibiotic residues in animal products, a screening programme exists to ensure they comply with set standards [27]. Strict legislation exists around residues of antibiotics in meat in food-producing animals within the EU. MRLs, the maximum concentration of a residue of a pharmacologically active substance that may be permitted in food of animal origin, are established according to Regulation (EC) 470/2009, with a view to public health and safety [5]. MRLs are listed in Commission Regulation (EU) No 37/2010 [6]. Monitoring of MRLs is the responsibility of individual countries within the EU, as is the establishment and implementation of a monitoring plan, according to the Commission Implementing Regulation (EU) 2022/1646 [6]. The requirements for the analytical methods that may be used in the testing of official samples and the criteria for the interpretation of analytical results are laid down in Commission Implementing Regulation (EU) 2021/808 [10]. The strategy for the control of residues is based on two steps: screening methods and confirmatory methods [17, 12].

There are many ways to test for antibiotic residues in food, and these can include microbiological assays, physical and chemical assays, immunoassays, aptasensors, and whole-cell biosensors [28]. Qualitative tests are used to determine whether a particular chemical is present in a sample. Quantitative tests are used to determine the absolute or relative abundance of a particular substance present in a sample. Qualitative tests are further divided into microbiological and immunological methods. Microbiological tests involve either test tubes or Petri dishes. Immunological tests involve ELISA, radioimmunoassay, and receptor-binding techniques [26].

In our study, initial screening for antibiotic residues in sheep meat sourced in Ireland was performed using the Explorer 2.0 tube microbial inhibition assay coupled with the e-Reader. Of the 15 randomly collected sheep meat samples, 2 samples were positive. When screening other samples from both positive suppliers, the sheep meat was already negative for antibiotic residues. Microbial inhibition tube tests are qualitative screening methods, which means that they give binary results (positive or negative). Even though these kinds of tests are ready to use and only require an incubator, they need to be halted at an adequate point in time to avoid over- or under-incubation and, thus, to obtain the best results in terms of sensitivity. Moreover, a visual reading of results can lead to misinterpretation, as well as discrepancies between lab results and those obtained by users [29]. The use of an e-Reader in conjunction with the Explorer 2.0 test proposed by Mata et al. (2014) [20] allows for the automation of the test. This device includes a thermostatic incubator adapted to the test tubes and an optical system to monitor colour change. Both are integrated with software that controls device functions and automatically detects the end-time of the assay to provide an objective result. Analysis can be performed at any location (on a farm or during lairage time at the slaughterhouse), even by untrained staff [21]. The manufacturer instructions specify that the ampoules cannot be interpreted based on the colour change following incubation in the e-Reader. With the cut-off value set to 56 units, the system reliably distinguishes positive samples from negative ones. The cut-off values of both positive samples were at or above the level of 56.

The two samples that tested positive using the Explorer 2.0 test were then tested using the STAR method. Inhibition zones greater than 2 mm on plates inoculated with Kocuria rhizophila ATCC 9341 and greater than 4 mm on plates inoculated with Bacillus stearothermophillus var. calidolactis ATCC 10149 confirmed the Explorer 2.0 test result and tentatively identified the antibiotic group in the examined samples. The test organism Kocuria rhizophila ATCC 9341 is set for the detection of macrolides and beta-lactams, and the test organism Bacillus stearothermophilus var. calidolactis ATCC 10149 is set for the detection of beta-lactams and sulphonamides. Two samples that tested positive with the Explorer 2.0 assay were subsequently tested with the STAR method. Zones of inhibition greater than 2 mm on plates inoculated with Kocuria rhizophila ATCC 9341 and greater than 4 mm on plates inoculated with Bacillus stearothermophillus var. calidolactis ATCC 10149 confirmed the Explorer 2.0 result and tentatively identified the antibiotic group in the samples studied. Based on the sensitivity of the test organisms to the respective antibiotic group(s), it can be predicted that of the two samples tested on plates with Bacillus stearothermophilus var. calidolactis ATCC 10149, only sample 11 was evaluated as positive for beta-lactams and sulphonamides, and on plates with the Kocuria rhizophila ATCC 9341, both samples were evaluated as positive for beta-lactams and macrolides. Because both test organisms are specific for ß-lactam antibiotics, we can assume a potential presence

of ß-lactam antibiotics in both sheep meat samples. For final confirmation of the result, whether the samples are positive for the presence of a beta-lactam antibiotic or a macrolide antibiotic or a sulphonamide, the screening result must be confirmed by subsequent confirmatory analysis.

Macrolides are effective against most aerobic and anaerobic gram-positive bacteria. They are used to treat systemic and local infections, such as upper respiratory tract infections, urinary tract infections, bronchopneumonia, and metritis. Sulphonamides are effective against both gram-positive, and gram-negative bacteria and some protozoa. They are used to treat systemic infections such as mastitis, metritis, respiratory infections, and toxoplasmosis. Beta-lactams are effective against gram-negative and gram-positive bacteria and are used to treat respiratory infections. Stricter measures surrounding veterinary prescriptions and record keeping came into effect on 28th January 2022 under Regulation (EU) 2019/6 [1], which strengthened food protection measures from a farming perspective.

The importance of the results that were found in this research shows that even though regulations and directives are in place on both a European and national level, there is still non-compliance in a small percentage of not only sheep but in other food-producing animals. Under the Article 19 of Regulation (EU) 2017/625 [7], each member state is required to implement a residue monitoring plan and to submit their programmes annually to the European Commission. The scope of testing under Ireland's National Residue Control Programme (NRCP) is comprehensive, covering 8 food production systems as well as milk, eggs, and honey and 19 distinct residue groups. These residue groups include prohibited substances having an anabolic effect and unauthorised substances (Group A) and authorised veterinary drugs and contaminants (Group B). In Group B, B1a relates specifically to aminoglycosides/antibiotics, B1b to insecticides/fungicides/anthelmintics/anti-parasitics, B1c to sedatives, B1d to non-steroidal anti-inflammatory drugs/corticorticoids/glucocorticoids, and B1e to other pharmacologically active substances.

The Department of Agriculture, Food and the Marine and local authorities in Ireland carry out extensive testing as part of the NRCP, which is then published in a report available on the DAFM website. The most recent report details residue monitoring for 2023, in which 2345 samples of meat from Irish food-producing animals were tested for Group B1a substances and 1591 samples for B1b substances. From 250 sheep/goat samples from Group B1a, no sample was positive; however, in Group B1b, 9 samples were positive. Of the 9 suspect samples that were confirmed to be non-compliant, closantel was detected in eight samples and levamisole in one sample. From other animal species, bovine meat was positive for eight antibiotics, one anthelmintic, four nitrofurans, and one thyrostat, and equine meat was positive for phenylbutazone and oxyphenbutazone [30].

As antimicrobial resistance is still a major global concern, it is vital that not only food producers but those working in the human health sector and the veterinary sector cooperate to ensure that antibiotics remain effective to treat illness for years to come. From this research it can be concluded that food needs to be safeguarded from residues of drugs, as a small percentage of food products can be overlooked in regard to testing random samples, as the consumer thinks that the food that they are buying is safe for consumption when it clearly is not.

CONCLUSIONS

The aim of our study was to determine whether there were antibiotic residues present in random sheep meat samples obtained from local sources in Ireland, using two microbial inhibition screening tests, the Explorer 2.0 test coupled to e-Reader and the STAR method. It is clear to see from the results gathered during the study that 2 of 15 samples tested positive for antibiotic residues using the Explorer 2.0 test coupled with an automatic system for reading and interpretation of positive and negative results. By further testing of both positive samples using the STAR method, it was found that based on the sensitivity of the test organisms of the STAR method, both samples were found to be positive for macrolides, beta-lactams, and sulphonamides. These antibiotics are commonly used to treat systemic infections in sheep. Although confirmatory analysis is required to declare a final result, microbial inhibition tests are and remain an effective tool in the screening of antibiotic residues in food of animal origin intended for human consumption.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

No ethical approval was necessary for this study.

Conflict of Interest

The authors declare no conflicts of interest.

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Authors´ Contributions

Conceptualization: I.K., D.H.; methodology: I.K.; sample collection: D.H.; investigation: S.H., D.H.; data collection: D.H.; data curation: I.K.; supervision: I.K.; writing – original draft preparation: D.H., I.K.; writing – review and editing: I.K. All authors have read and agreed to the published version of the manuscript.

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