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# Detection of Antibiotic Residues in Sheep Liver at Almoilih Slaughter Houses at Karrey Locality, Khartoum State, Sudan

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**A B S T R A C T**

The objective of this study was to detect antibiotic residues in sheep liver collected from Albaraka and Alsabaloga slaughterhouses at Karray locality in Khartoum state. A total of 28 sheep liver samples were taken to microbiology laboratory, microbial inhibition one plate of *Bacillus subtilis* in agar medium was used. The results revealed that 7 (25%) of sheep livers were positive to antibiotic, while 21 (75%) of them were negative. In conclusion the use of antibiotics in food producing animals' must be under control; it should be given at recommended dose with appropriate supervision. Adequate holding period or withdrawal period should be observed in all slaughtered animals following therapeutic use of antibiotics in treating sick animals, in addition to continuous training veterinarians to know the importance of withdrawal period in avoiding tissue residues.

## Introduction

In developed countries the uses of antibiotics in animal husbandry, whether as prophylactic or therapeutic agents or as growth promoters in feeding stuffs are needed to restrict the potential problems resulting from the misuse and extra-label (Report, 1969). Veterinary drugs residues usually accumulate in the liver or kidney rather than other tissues. It has been noted that different residue levels can be found in different tissue positions such as site and route of administration (Doyle, 2006).

Residues from these substances are present in edible tissues milk and eggs and may exert different levels of toxicity on consumers upon consumption (Suhren *et al*., 1996), with anemia, hypersensitivity and resistance to antibiotics. Screening of animal products for veterinary drugs began mainly with the dairy industry to overcome problems related to fermentative dairy production, from 1970s regulatory screening of slaughtered animals was started (Mariël, 2007) to prevent or minimized the harmfully effect of the residues. Microbiological techniques are the

basis of screening methods for monitoring the presence of veterinary drug residues, which possess antibiotic or antibacterial activity in foods of animal origin (Bogaerts and Wolf, 1980). Screening methods have acceptable false-positive result rates (Heitzman, 1992; Korsrud and MacNeil, 1987) and allow detection of a wide spectrum of antibiotics (Aerts *et al.*, 1995; Haasnoot *et al.*, 1999). These methods use liquid or solid media inoculated with a standard culture of test microorganisms, e.g. *Geoba cillus* stearothermophilus var. calidolactis C 953, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, *Bacillus megatherium*, or *Streptococcus ther-mophilus* (Botsoglou and Fletouris, 2001; Heeschen, 1993).

The detection methods based on microbial inhibition are able to detect a wide range of antimicrobials, very cheap, easy to apply but they are lack of specificity when compare with the chemical methods, such as HPLC and GC-MS are usually sensitive enough to comply with maximum residue limits (MRLs) but are too specific and expensive for routine screening purposes (Bogaerts and Wolf, 1980).

The four-plate test (4PLT) has been recognized in Europe as a valuable method for the detection of antibiotic residues. However it was designed for meat and the use of four different agar diffusion plates makes it rather expensive and time consuming. This paper describes a one-plate screening method (OPS) for antibiotic residues in slaughter animals that has been used in Belgium for EEC screening purposes.

The method is based on the microbiological detection of antibiotic residues by growth inhibition of *Bacillus subtilis* in agar medium (Wenzel, 1982). The aim of the paper was to detect and identify the antibacterial residues in sheep liver and to test bacterial growth inhibition.

## Materials and Methods

### Study area

The samples were collected from slaughter houses at Almoilih aera in Karrey Locality, Khartoum State in January 2019.

### Sample size

Twenty eight sheep liver samples were selected randomly from slaughter houses according to Non-probability sampling methods (Thrusfield, 2007).

### Sample collection

The 28fresh slaughtered sheep liver samples were transported to the laboratory of Microbiology at the College of Veterinary Medicine in Khartoum. Then15 grams were taken from each liver. The samples were put in plastic bag, labeling and stored in ice keeper.

### Preparation of samples

Samples were removed from the deep-freeze and allowed to reach a temperature of about -5 'C, before the outer (contaminated) surface was removed with a sterile scalpel. A cylindrical piece of liver was removed from each sample using a sterile cork borer (8 mm internal diameter), 2 mm thick, were cut from it. The *B. subtilis* plates were incubated at 30ºC for 18-24 h. A positive test result was recorded when inhibition zone not less than 1-2 mm across.

### Antibiotic detection

The One plate Screening method (OPS) was used *Bacillus Subtilis* DSM618.

## Preparation of test plates with *B. subtilis* CCM 4062

The pH 6 test agar was heated to 55 °C and inoculated with *B. subtilis* spore suspension to approximately 104 CFU $ml^{-1}$. The agar with the test strain was pipetted at 4 ml doses to pre-heated sterile glass Petri dishes of 90 mm in diameter.

## Culture medium

Standard II nutrient agar (Merck 7883) was prepared with 0-4% (w/v) dextrose. The medium was sterilized by autoclaving at 121°C for 20min. The pH was adjusted to 7 ±00. 5 with IN NaOH and IN HC1. When the medium had cooled to 50°C, trimethoprim at a concentration of 0-2 /ig/ml agar and a spore suspension (Merck 10649) containing 104 *Bacillus subtilis* BGA spores per ml were added. Fourteen ml of the culture medium was poured into 9 cm diameter petri dishes.

The solidified agar medium was then kept upside down in the refrigerator at 4°C in plastic bags. The plates were used between 1 and 7 days after preparation (Koenen – Dierick *et al.,* 1995). An incision was made into the liver sample to have around 0.5 gram in 5 mm thick to place immediately into the Petri dish.

## Cultivation media and solutions

In order to prepare test plates with *B. subtilis*, test agar pH 6 was used. Further, sporulation medium was used (containing, in 500 ml, proteose peptone 1.725 g (HiMedia), casein enzyme hydrolysate1.725 g (HiMedia), NaCl 2.55 g; Agar No. 1.

6.5 g (Oxoid, 2006); potassium dihydrogen phosphate 0.5 g (KH2PO4, Merck, Darmstadt, Germany) pH 7, sterilized at 121 °C for 15 min.

## The principle of method

The principle of the test is preparing plates seeded with sensitive bacteria (*Bacillus subtilis*) at specific conditions that can presumptively indicate the presence of specific antimicrobial group residues. The samples can be applied on top of, the agar layer. After over-night incubation, the presence of an antimicrobial residue becomes visible as an inhibition zone around the sample. The size of the inhibition zone depends on the type of residue and its concentration, while the sensitivity of the test is affected by many factors, such as indicator organism, pH, type of growth medium, and thickness of the agar layer (Bovee and Pikkemaat, 2009).

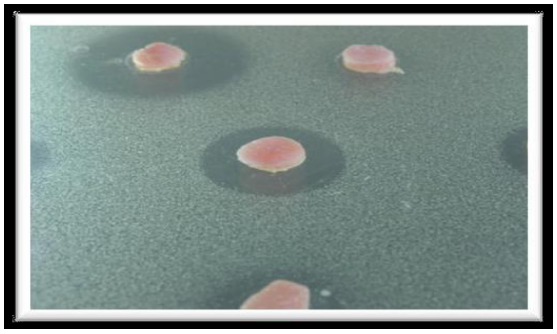

**Figure.1** Muscle samples on a plate of FPT (Pikkemaat, 2009)

## Preparation of *B. subtilis* spore suspension

The *B. subtilis* spore suspension was prepared in accordance with the method of Bogaerts and Wolf (1980).

## Factors may influence sensitivity results of agar diffusion methods

A number of factors may influence sensitivity results of agar diffusion methods besides the type of the test organism. It has been reported that the results might be affected by, e.g., the composition of the sample tested, agar

thickness, concentration of the test strain spores in the medium, technique of sample application, agar pH, evaluation of results, etc.( Pavlina *et al.*, 2010)

**Data analysis**

The data was analyzed by using SPSS (Statistical Package for Social Science) version 24.

## Results and Discussion

The results in Table 1 and Figure 1 about 25 % of the sheep liver samples were positive to antibiotics while 75% was negative. Relationship between the percentage of positive inhibition zone (IZ) higher than 2mm or less than 2 mm and negative IZ < 2 mm the analysis concentration was calculated, from which the calculations were made of the concentration threshold value at which the tests become unreliable and, at the same time, a concentration to which the given strain is sensitive. When evaluating results in individual Petri dishes, they measured the diameter of the inhibition zone (IZ) of all samples there, and then they determined the mean diameter of IZ in mm. A sample was considered as positive when the mean diameter of IZ thus calculated was ≥ 2 mm. The method's sensitivity to an antimicrobial substance was defined as the lowest concentration at which a positive result was obtained. However in the research, a method was considered as sufficiently sensitive at a given antibiotic concentration when a positive result, i.e. IZ ≥ 2 mm, was obtained with all the samples (Pavlina *et al*., 2010). This result is closed to the study conducted with (Hind *et al.*, 2014), he found a total of 221poultry tissue samples screened for antibiotic residues were 27% of the samples tested positive residues and 73% were negative. Also this result similar to the study of (Shahid *et al*., 2007) done in Pakistan using *B. subtilis* as a test organism, screening of AMR in a total of 100 broiler tissue samples (33 livers, 33 kidney and 33 muscles) revealed that 13(39.4%) livers, 9(27.3%) kidneys and 7(20.6%) muscles contained antimicrobial residues.

**Table.1** The distribution of antibiotics in the sheep liver

| Value | Frequency | Percentage |
|---|---|---|
| **Positive** | 7 | 25.0 |
| **Negative** | 21 | 75.0 |
| **Total** | 28 | 100.0 |

The microbiological methods for screening the antibiotics it's still useful and coast less for monitoring the antibiotics in animal products especially for the large herds in developing countries to avoid the harmful residues to human and animal's health.

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