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# Detection of Residual Antibiotics in Calf Meat and Kidney Employing *Bacillus subtilis* Disc Assay

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Sixty five (65) meat and kidney samples that were belong to the same calf, from different sources were analyzed for antibiotic residues. Detection of residual antibiotics in calf meat and kidney were made by *Bacillus subtilis* disc assay method. Data showed that 1.53 % of the meat and kidney results were positive. The research indicated an urgency to advocate rational use of antibiotics in animals for safeguarding the human health and the enforcement of standards as important in protecting public health.

**Key words:** Antimicrobial residues, calf meat and kidney, *Bacillus subtilis*

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## Introduction

Most of the better-known antibiotics have been tested on raw foods, chiefly proteinaceous ones like meats, fish, and poultry, in an endeavor to lengthen the storage time at chilling temperatures. Aureomycin (chlortetracycline) has been found superior to other antibiotics tested because of its broad spectrum of activity. Terramycin (oxytetracycline) is almost as good for lengthening the time of preservation of foods. Some success also has been claimed with chloromycetin (chloramphenicol). These three antibiotics inhibit protein synthesis in the cell. Streptomycin, neomycin, polymyxin, nisin, subtilin, bacitracin, and others are not as satisfactory, and penicillin is of little use. Nisin has been employed in Europe to suppress anaerobes in cheese and cheese products. Natamycin is effective against yeasts and molds; it is used, or tested, in orange juice, fresh fruits, sausage, and cheese (Frazier and Westhoff, 1988).

Experimentally, antibiotics have been combined with heat in attempts to reduce the thermal treatment necessary for the preservation of low- and medium-acid canned foods. Most tests have been applied with the peptides, subtilin, nisin, and tyrosine. It has been suggested that a botulinum cook, i.e., enough of a heat treatment to inactivate all spores of *Clostridium botulinum*, be given canned foods, combined with the addition of enough antibiotic to inhibit germination and outgrowth of surviving spores of the most heat-resistant thermophilic spoilage bacteria and putrefactive anaerobes. Subtilin supposed has no effect on the heat resistance of bacterial spores but inhibits heat-damaged cells during outgrowth, whereas nisin apparently interferes with spore germination and with lysis of the spore coat. Tyrosine may inhibit cell growth (Frazier and Westhoff, 1988; Campbell and O' Brien, 1955; Farber, 1959; Goldberg, 1964).

Although food bacteriologists realize the advantages of the preservation of raw foods by a nontoxic antibiotic or the use of one in combination with reduced amounts of heat in the processing of canned foods, they raise certain questions about the use of antibiotics as preservatives. They agree that antibiotics never should be substituted for good hygiene. The effect of an antibiotic on micro-organisms is known to vary with the species or even with the strain of the organism; hence the antibiotic may be effective against some spoilage organisms but not others or against part of the population in a culture but not all organisms. Organisms are known to become adapted to increasing concentrations of an antibiotic so that new, resistant strains may develop. It has been recommended that antibiotics selected for use in food preservation be other than those being processing of canned foods, the antibiotic plus the heat treatment must destroy all spores of *C. botulinum* and allow a margin of safety, and preferably the treatment should destroy all spoilage organisms and their spores. If spores survive, the antibiotic must remain in sufficient concentrations in the food to prevent germination, outgrowth, or vegetative growth of cells. This means that the antibiotic must persist in bacteriostatic or sporostatic concentration throughout the storage life of the canned food (Frazier and Westhoff, 1988; Campbell and O'Brien, 1955; Farber, 1959; Goldberg, 1964).

The discovery and wide use of antimicrobial agents have had a recognizable and profound impact on the life and heath of human beings and animals. By controlling infectious diseases and promoting health, these drugs have greatly improved life for the individual and for society at large (Nichols and Keys, 1984).

The aim of this investigation was to detect the antibiotic residues in the meat and kidney of calves.

## Materials and Methods

The research work was conducted at Food Science and Technology Division, KSU Agriculture Faculty, Turkey, during 1st Feb to 29th May, 2001.

**Meat and kidney samples:** Sixty-five muscle and kidney bovine samples that were belong the same calf were obtained from different markets and samples were stored at the laboratory at -20 °C for an average of 12 days until tests were completed.

**Detection of antibiotic residues:** This was determined by *B. subtilis* disc assay (İnal, 1992, Anonymous, 1987). This test is based on inhibition of the growth of *B. subtilis*. A clear zone of 2 mm inhibition is considered as a positive test, 1-2mm is considered as a doubtful test in a disc assay. However, the diameter of the inhibition zone must be more than 6 mm (İnal, 1992). The absence of clear zone in disc assay for detecting antibiotics means that the meat and kidney is either free of antibiotic residues or that the amount present is less than the detectable levels of the test, the organism of choice being *B. subtilis* (İnal, 1992; Anonymous, 1987).

The tissue and kidney were cut into small pieces (8 mm in diameter, 2 mm high). Meat and kidney samples were applied on the Mueller Hinton Agar (Difco Lab. Detroit- Michigan) which were adjusted at pH 6, 8 and 7 (as control) value by pressing slightly. The medium was incubated at 30 ± 0.1 °C for 18-24 hours, and 0.001 IU penicillin test kit (Merck) which was at pH 6 and 0.5 mg streptomycin test kit (Merck) was added to the medium which is at pH 8. At the end of the period, inhibition zones formed on the medium were measured with a transparent ruler in mm and compared with the reference drugs.

For controlling the negative results, resistance of the test bacteria was checked by standard antibiotic discs, such as ampicillin (Oxoid, 10 μg), cefodizime (Oxoid, 30 μg), cefuroxime (Oxoid, 30 μg), cephalothin (Difco, 30 μg), tarivid (Ofloxacin) (Hoechst, 10 μg), oxacillin (Oxoid, 1 μg), tobramycin (Difco, 10 μg), and vancomycin ( Difco, 30 μg), used for comparison, were provided by the Microbiology Division of the Medicine, Faculty of KSU, Turkey.

**Test bacteria:** *Bacillus subtilis* IMG 22 were used in this investigation and provided by the Microbiology Division of the Science and Arts Faculty, Biology Department of KSU.

*B. subtilis* streaked on the medium mentioned above (pH 7 ± 0.2) was incubated at 30 ± 0.1 °C for 10 days. The organism that were grown on agar slopes washed off in physiological saline and centrifuged at 3000 rpm for 10 min and then the supernatant and the physiological saline were heated at 70 °C for 30 min. The density of the suspension was adjusted $10^7$ bacteria per ml (İnal, 1992).

## Results and Discussion

The results (Table 1) of screening and confirmatory assays of antibiotic residues from calf meat and kidney samples showed that one of 65 calf meat sample (1.53 % of the meat) and one of the 65 kidney sample was (1.53 % of the kidney) positive. The positive kidney sample came from the animal that was given the same positive meat sample. The zone of inhibition (ZI) around the calf meat was 14 mm, and around the kidney was 16 mm.

The inhibition zone formed by standard antibiotic discs against *B. subtilis* is resistant to three of the used antibiotics (Table 2).

A study was conducted in five volunteers who prepared, cooked, and ate chickens from which drug-resistant *Escherichia coli* had been isolated and serotyped. Fecal specimens from the volunteers were examined before, during and two weeks after handling the birds. The same serotype of *E. coli* was found in one volunteer as in the chickens. Handling contaminated poultry was found to be the most prevalent means of transmission to humans (Linton, 1977). In one study of personnel on a poultry farm in New England, within 5 months after the poultry were started on subtherapeutic oral tetracycline, the prevalence of tetracycline resistant bacteria in the intestinal flora of persons living on the farm was more than 10 fold higher than neighbors that were not in contact with animals on medicated feed (Levy *et al.*, 1976; Anonymous, 1980). Questions and discussions concerned with the potential public health hazards from subtherapeutic feeding to animals have been debated for nearly 35 years. Research difficulties involved in specifically associating human heath

Table 1: Results of antibiotic residues from calf meat and kidney from Kahramanmaraş city

| Type of meat | Type of tissue | Inhibition zone of diameter (mm) | % of positive sample for antibiotic residue |
|---|---|---|---|
| Calf | Muscle | 14 | 1.53 |
| | Kidney | 16 | 1.53 |

Table 2: The inhibition zones formed by standard antibiotic discs against *Bacillus subtilis*

| Diameter of inhibition zone (mm) of standard antibiotics | | | | | | | |
|---|---|---|---|---|---|---|---|
| Amp 10μg | Cef 30μg | Cefu 30μg | Cep 30μg | Oxa 1μg | Tob 10μg | Oflo 10μg | Van 30μg |
| 10 | - | 13 | - | - | 24 | 35 | 20 |

Amp: Ampicillin, Cef: Cefodizime, Cefu: Cefuroxime, Cep: Cephalothin, Oxa: Oxacillin, Tob: Tobramycin, Oflo: Ofloxacin, Van: Vancomycin

problems to meat animals fed antibiotics persist (Anonymous, 1980).

Attempts have been made to test the bactericidal effect of edible extracts of plants, e.g., carrot, green bean, tomato and celery plants, in combination with a milder heat treatment than usual, to destroy various bacteria and bacterial spores. The use of plant extracts in this manner would avoid most of the problems just discussed. Although in America the only permitted use of antibiotics in flesh foods now is in fish (chlortetracycline at 7 ppm in ice), experiments have indicated that antibiotics can be used successfully in meats to prolong storage life at chilling or higher temperatures. The antibiotics most often recommended have been chlortetracycline, nisin, and chloramphenicol. The antibiotics may be applied to meats in various ways:

a The antibiotic may be fed to the animal over a long period
b It may be fed more intensively for a short period before slaughter
c It may be infused into the carcass or into parts of it
d It may be applied to the surface of pieces of meat or mixed with comminuted meat.

Feeding an antibiotic brings about a selection of microorganisms in the animal's intestinal tract, presumably reducing the numbers of spoilage bacteria there and therefore reducing the numbers that are likely to reach the meat from that source during slaughter and dressing. It has been suggested that injection of antibiotic before slaughter might be employed to prolong the keeping time of carcasses at atmospheric temperatures before they reach the refrigerator to hold beef briefly at temperatures that will favor tenderization of special cuts as well as lengthen the keeping time of meats held at chilling temperatures. Infusion of an antibiotic into the carcass immediately after slaughter or into special parts would serve similar purposes. The storage life of meats could be lengthened by means of an antibiotic dip or by inclusion of an antibiotic in ground meats (Philips *et al.*, 1961). The subtherapeutic use of antimicrobial drugs has played an important role in animal husbandry by the control of disease, the improvement of growth and efficiency of feed conversion. Livestock producers, industry, veterinary, and regulatory personnel share responsibility to ensure freedom from drug metabolites, residues, and other chemicals to which livestock and poultry may be exposed.

Therefore this investigation indicated that *B. subtilis* disc assay method can be used for qualitative estimation of antibiotic residues in meat and kidney. Use of antibiotics in human and veterinary medicine has been implicated as adding to the problem of drug resistance in bacteria. Un-needed antibiotics prescribed to a stressed patient, whether human or food animal, allow growth of antibiotic-resistant organisms which can multiply to the detrimental effect of the host. Veterinary, as well as human medicine, should assume the responsibility for eliminating these uses. If ethical and professional judgement were exercised, perhaps the need for governmental restriction or prohibition would decrease, the situation would improve, and the public health would be safeguarded.

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