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Overview of Antibiotic Residues in Beef and Mutton in Ardebil, North West of Iran

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Abstract: Due to their availability and low cost, antibiotics have been widely used in the dairy, livestock, poultry, aquaculture and honey production industries in various countries around the world. Antibiotics are natural products of a micro-organism, identical synthetic products or similar semi synthetic products that inhibit the growth of or destroy microorganisms. In veterinary medicine antibiotics are used for therapeutic, prophylactic, metaphylactic and nutritive purposes. The presence of antibiotics or their metabolites in food is potentially hazardous to health as it may cause allergic reactions in people and antibiotic resistance in pathogenic microorganisms. In addition antibiotics may influence starter cultures in food industry and lead to economic damage. The methods used to detect antibiotic residues in food of animal origin are microbiological, immuno-enzymatic and chemical. Microbiological methods are used as screening methods whose results direct the choice for more expensive and time consuming chemical confirmation methods. This is due to concerns about possible carcinogenicity and mutagenicity but for public little is known about food safety in relation to antibiotic residues. A total of 500 samples of beef and mutton were screened for drug residues. Finally the prevalence rates of drug residues in the various animal source foods were; 22.8% (beef) and 14% (mutton). Considering the relatively high contamination rates of animal originated food as well as their high rates of consumption, it is likely that consumers experience a high risk of exposure to drug residues, especially through beef. Specific causal factors associated with non-adherence of withdrawal requirements of drugs and therefore contaminations of food were mainly knowledge related factors. This underscores the importance of health education of farmers in dealing with the problem of drug residues contaminating animal source food.

Key words: Antibiotic residues · Beef · Mutton · Iran · Ardebil

INTRODUCTION

Concerns about food safety especially, with animal originated food are increasing in developing countries where urbanization, increasing incomes and changing of life-styles are associated with greater dependence on marketed foods by an increasing number of people [1]. The safety of human food is threatened by various agents including pathogenic microorganisms, aflatoxins, pesticides and antimicrobial agents. Pathogenic microorganisms constitute the most important food related threat to public health. Relatively, little is known about food safety in relation to antimicrobial agents, in the developing world. While pasteurization and other forms of heat treatment eliminate pathogenic microorganisms from animal source food, these procedures have limited or variable effects on drug residues in animal originated food [2]. Various antibiotics used in the treatment of animal diseases have been shown to occur in animal products used as human food [3] and are usually attributed to non-observance of withdrawal periods before sale of animal source food [4]. Additionally, the drugs may be introduced through the use of antibiotics in animals for therapeutic and growth purposes [3]. Behavioral practices such as overuse of drugs and lack of understanding about drug usage also contribute to food contamination. The presence of antibiotics in human food is associated with several adverse public health effects including hypersensitivity, tissue damage, gastrointestinal disturbance and neurological disorders [3, 5]. Additionally, the use of antibiotics in animal husbandry and its occurrence in

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related food, may lead to selection of resistance in bacterial populations that do not respond to treatment commonly used for human illnesses [5]. Antibiotics can also be classified as broad or narrow spectrum, depending on the range of bacterial species against which they are active, or as bacteriostatic or bactericidal on the basis of their mechanism of action. The latter fall into four categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function and inhibition of protein synthesis. The aim of anti microbiological therapy is to rapidly produce and then maintain an effective concentration of drug at the site of infection for sufficient time to allow host specific and nonspecific defenses to eradicate the pathogen [6]. Antibiotics are administered to animals by injections (intravenously, intramuscularly, or subcutaneously), orally in food or water, topically on the skin and by intramammary and intrauterine. infusions [7]. Theoretically, all these routes may lead to residues appearing in foods of animal origin such as milk, meat and eggs [8]. Acquisition of resistance to antimicrobial agents by consuming food of animal origin has been receiving increasing attention in the literature, also raising awareness of the importance of minimizing exposure to antibiotic residues in food [1, 8]. The most common causes for the presence of antibiotic residues in food of animal origin are violation of withdrawal periods, overdosing of antibiotics and use of antibiotics banned for treatment of economic animals [5, 10-12]. Microbiological, immuno-enzymatic and chemical methods are used for detection of antibiotic residues in food of animal origin and the protocol of control is usually based on two steps: screening for presence of different antibiotic groups and confirmation with identification of specific antibiotic in the sample and more accurate quantitative analysis.. An ideal screening method would detect all licensed antibiotics at or below their Marls and should be robust, rapid, simple and cost effective [13]. Microbiological methods detect inhibitory substances diffusing from a piece of tissue [2, 14, 15] or from a paper disk soaked with tissue fluid [4] into an agar layer seeded with a susceptible bacterial strain. The most commonly used antimicrobials in food-producing animals are ß-lactams, tetracyclines, aminoglycosides, quinolones, macrolides and sulfo-namides. A study by Aning *et al*. [16] on raw milk indicated that antibiotics may be translocated at high rates into raw milk, though the study did not elaborate the associated causal factors. Currently, there are no exact data about rate of this substance at consumed meat and neither major quality assurance programmes in place in the country to protect public health against the adverse effects of antibiotics used in animal husbandry, which is partly due to the lack of research data to inform policy. This study was carried out to help/address these needs and to provide fundamental data for future surveillance of these public health hazardswith.

METHODS AND METHODS

The Study Area: The study was carried in the City of Ardebil that it located in Northwest of Iran. The climate in the study area is cold and humid; the weather began to cool down from September to mid-April. The minor rainy season starts from early September and Rainy season is mainly associated with snow. The mean daily temperature is 14±3C. The relative humidity can be as high 87±5% on most days of the year.

Sampling and Data Collection: The study was carried out from January, 2010 to November, 2011. A stratified, purposive and random sampling methodology was used. Retail stores were randomly selected to cover two types of meats including, cattle and sheep. Overall, a total of 500 samples comprising 250 beef and 250 mutton were collected in the study so sampling was carried out at retail stores, where meat samples of about 50 g of different animals were collected and frozen until they were ready to be analyzed. This comprised 500 meat (muscle tissue) samples; no organ meats were sampled and background information of the animals was not available.

Laboratory Analyses: Animal originated food samples were screened for antibiotic residues using the microbial inhibition plate test described [11] but with some modifications in terms of the antibiotics used as controls. The test detects antibiotic levels above recommended maximum residue for various families of antibiotics including b-lactams, tetracyclines, chloramphenicol, macrolides, aminoglycosides, sulphonamides and quinolones. Briefly, the test procedure is described as follows: using Bacillus subtilis BGA strain as the test organism, a 0.5% McFarland's standard suspension of the organism was prepared. This was used to inoculate the surface of Mueller-Hinton agar plates prepared at pH ¼ 7.0 and containing 0.2 mg/ml of trimethoprim. A sterile 8 mm diameter cork borer was used to create disc shaped meat samples of 2 mm thickness, which were applied to the surface of the agar medium.. Positive controls were set up with 1 mg/ml of ciprofloxacin, chloramphenicol and

tetracycline, while negative controls were set up with distilled water. The agar plates were incubated at 37°C for 18e24 h. After incubation a zone of inhibition of 1 cm or more was considered a positive case of the meat sample containing drug residues. Positive controls were expected to have zones of inhibition while the negative control was not expected to have any zone of inhibition [11].

Data Analyses: Data were analyzed using one way analysis of variance. Means were compared using Tukey HSD test, all statistical analyses were performed with SPSS soft ware version 17.

RESULTS

Many types of antibiotics are routinely used for therapeutic and prophylactic purposes in farm animals. Anabolic compounds are used mainly in beef cattle for promoting growth but are not widely used in sheep. Many methods have been used in the past to detect hormonal residues in meat including: uterus weight bioassay, prostate test, thin layer chromatography in urine. However, there are not official detection reports of residues in the Ardebil area. So our result may be useful for control and provide condition for more studies. A total of 500 samples were collected for antibiotic residue analysis. The overall prevalence of drug residues in Animal originated food was 18.4%. The prevalence rates of drug residues in the various animal originated foods varied by type and location (Table 1). In beef and mutton, prevalence rates were 22.8% and 14% respectively. Beef showed the higher prevalence rate of drug residues at compared with mutton. Significant differences were observed in the prevalence rates of antibiotic residues among the different types of meat at ($p<0.05$).

Risk of Consumer Exposure to Antibiotic Residues: The overall food contamination of 18.4%. with drug residues translates into an average risk of exposure every five time a consumer takes in animal source food. However, the actual risk of exposure associated with the different animal originated food types would differ due to differences in contamination rates with drug residues and/or consumption rates. The highest overall food contamination rate of 22.8% which was associated with beef translates into an average risk of exposure one in every four time a consumer takes in the commodity (beef). Considering the relatively high contamination rates of the various meat types and the fact that the drug screening test used, detects drug residues above maximum residues limits, it is likely that consumers experience a high risk of exposure to drug residues through meat.

Table 1: Numbers of different animal originated food sampled and their prevalence rates of antibiotic residues

	Food type		
	NT	NP	%P
Beef	250	57	22.8%
Mutton	250	35	14%

NT, total number of samples tested.

NP, number of positive samples.

%, proportion of positive samples.

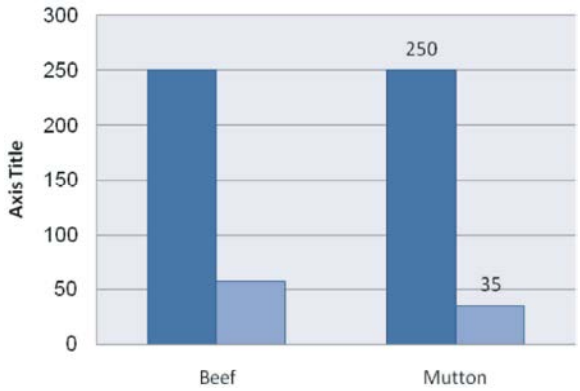

Fig. 1: Show the number of cases with antibiotics residues in beef and mutton

Factors predisposing to contamination of animal source food with antibiotic drug residues:

- Adherence to withdrawal requirements of antibiotics by farmers which was assessed
- Knowledge of withdrawal requirements of drugs
- Knowledge of possible health risk associated with drugs in animal source food
- Consultation of a veterinary officer in drug usage
- Previous training in animal farming
- Farm type (whether intensive or extensive farming)

Lack of consulting a veterinary officer and lack of knowledge of withdrawal requirements of drugs, as well as health risks associated with drugs in animal source foods were risk factors for non adherence to withdrawal requirements of drugs. Similarly, extensive farming practice and lack of previous training in animal farming were risk factors for non-adherence to withdrawal requirements of drugs.

DISCUSSION

The sensitivity of a microbiological method must be high enough to allow detection of antibiotic residues level below the MRL prescribed in the current legislation (33). In some of the already published studies only working solutions were used instead of the real matrix investigated in routine analyses. This may lead to falsely low levels of detection. According to our experience the influence of matrix on the results should not be neglected and this is especially true in the case of meat as a solid matrix whose preparation is especially troublesome. For this reason the recovery should always be calculated. In several Scandinavian countries kidneys are used as the matrix from which the level of antibiotics in meat is assessed, because the concentration of antibiotics and therefore the MRL are higher in kidney tissue compared to meat. In present our study we identified b-lactame, aminoglicoside, macrolide, tetracycline and qionolone antibiotic groups at meat. Consumer risk of animal source food in Iran and several other developing countries is largely unknown. There are currently very limited studies on the subject and in Iran the available data focus on raw milk, a commodity rarely consumed in the country. In this study, the prevalence rates of drug residues in the two types of meat specimens ranged from 14 to 22.8%. The prevalence of drug residues in meat specimens is generally similar to the rate reported in milk (25.5%) by Aning *et al*. [16]. High rates of drug residues in various animal source foods have been reported in several other countries especially, in the developing world such as Tanzania [12] and Kenya [10]. Generally, in the developed world, drug residue contamination of animal source food is low and in Europe prevalence rates of <1% are generally reported [8]. The microbial inhibition plate test used to screen food for drug residues has a low sensitivity for macrolides but has a high sensitivity for many other antibiotics such as b-lactams, tetracyclines, aminoglycosides, sulphonamides and quinolones [11]. Though the test detects a wide range of drug residues in food above maximum residue limits, it is unable to differentiate and quantify the residues. The risk of exposure of a human population to food contaminated with antimicrobial drug residues depends mainly on the extent of food contamination and the consumption rate of the food [13, 17, 18]. In this study, because it was not possible to quantify specific drugs in the food samples, it is difficult to measure the actual risk of specific drugs to consumers. However, considering the relatively high contamination rates of animal source food as well as their high rates of Consumption, it is likely that consumers experience a high risk of exposure to drug residues, especially through beef. Various forms of health risks associated with drug residue contamination of food have been reported but the most common appears to be allergic reactions [7, 15]. In Iran, both drugs are known to be used on animals, but penicillin is more frequently used. As determined by the Codex Alimentarius Commission, the acceptable daily intake of penicillin is 30 mg/ kg body wt, while that of sulphonamide is 50 mg/kg body wt [13, 18]. Penicillin may be deemed to be the most problematic antimicrobial agent in Iran as far as health related risk of drug residues in animal source food is concerned. This is due to its frequent usage in animal husbandry, its relatively low acceptable daily intake and the susceptibility of a large proportion of people to penicillin as an allergic trigger. It is encouraging that majority of the consumers are aware of the possible transfer of antibiotics into animal source food and the associated health risks. However, this is not likely to impact positively on the safety of animal source food, as consumer practices of heat treatment tends to have no effect on antimicrobial agents. Preventing contamination of the food is the key food safety measure and this is dependant mainly on drug handlingpractices of farmers in animal husbandry. Non-observance of withdrawal requirements of drugs by farmers and related factors underlie the contamination of animal source food with antibiotic drug residues [4, 19]. Five main factors were significantly associated with adherence to withdrawal requirement of drugs. Owing to the direct relationship between contamination of animal source food with drug residues and non-adherence of withdrawal requirement of drugs, these factors represent risk factors of food contamination. Interestingly, most of the risk factors were related to lack of knowledge of farmers including, knowledge about withdrawal requirement of drugs, health risk of drugs in animal source foods, as well as knowledge gained from previous training in animal handling. This underscores the importance of health education of farmers in dealing with the problem of drug residues contaminating animal source food and the associated public health hazards. Proper veterinary consultation by animal farmers has been reported to control several veterinary public health hazards [6, 14]. Thus it is not surprising that lack of veterinary consultation by farmers in drug administration was identified as a risk factor for food contamination with drug

residues. The significant association of extensive farming (moving animals from one place to another in order to graze) with drug contamination of food is difficult to explain. However, it is likely that in extensive farming, reduced control of animals makes it difficult to efficiently monitor animals after drug administration.

False positives are more likely to occur with kidney samples rather than meat which were assayed in this study. This study has exposed a potentially serious public health problem for consumers of locally produced animal source food in Iran. Two recommendations to begin to address this problem are: (a) creation of greater awareness among policy makers and design of potential interventions, (b) testing for drug residues at meat distribution centers. Socio-economic analysis of non-compliance with withdrawal requirement of drugs would be required to understand the potential incentives that would lead to behavioral change among farmers. Additionally, there is the need for studies concerning animal feed that are mixed with antibiotics.

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