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ORIGINAL ARTICLE

Determination of antibacterial residues in raw cattle beef in some selective areas of Mymensingh District during Eid-ul-Adha

M. Comruzzaman, A.K.M.H. Kabir, A. Nahar, N. Hassan, M.M. Alam*

Department of Medicine, Bangladesh Agricultural University, Mymensingh 2202

Abstract

Background: Antibiotic residue in foods of animal origin is a growing public health concern in recent time. Indiscriminate use of antibacterials in food animal production and ignoring proper withdrawal period may lead to drug residue in milk, beef and eggs. The present study was conducted to detect and quantify antibacterial residues in raw cattle beef samples.

Methods: One hundred cattle beef samples were collected from four Upazillas of Mymensingh named Gofargaon, Fulpur, Dhobaura and Bhaluka, Bangladesh during the period of Eid-ul-adha in 2018. The residue of two most commonly used antibacterials named oxytetracycline (OTC) and sulphadimidine (SDI) were quantified by using High Performance Liquid Chromatography (HPLC).

Results
The mean concentration of OTC and SDI residues in raw beef samples were 0.145 µg/ml and 0.094 µg/ml and the concentration ranged from 0.104-0.435 µg/ml and 0.083-0.357 µg/ml, respectively. About 59% and 43% beef samples contained some levels of OTC and SDI residues, respectively. Importantly, 19% and 12% beef samples exceeded maximum residue limit (MRL) for OTC and SDI, respectively.

Conclusions: The results indicate that drug residue is common in raw beef samples and a good proportion of beef samples exceeded MRL. Farmer's education to ensure rational use of antibacterials and to follow withdrawal period may help control this public health hazards in Bangladesh context.

Keywords: Withdrawal period, Oxytetracycline, Sulphadimidine, MRL, HPLC

*Correspondence: asamahbub2003@yahoo.com

Introduction

Antibiotic resistance (AMR) has gained a global health concern. A recent scientific review indicates that if no solutions are found, infections caused by AMR pathogens will lead to around 700,000 deaths in a year and are predicted to cause 10 million deaths annually by 2050 (WHO, 2016). Extensive use of antimicrobials as therapy and prevention of human and animal diseases as well as antimicrobial growth promoters (AGPs) in food animals and subsequent release of these drug residues through milk, beef and egg is a leading cause of development of antibiotic resistance in the human body (Woolhouse *et al*., 2015). AGPs are antibiotics added to the feed of food animals to enhance their growth rate and production performance. The mechanism by which AGPs work is not yet clear. However, AGPs reduce normal intestinal flora and harmful gut bacteria, which might have the effect on growth (Wegener *et al.* 1999). The most common antimicrobials used as therapy and AGPs in food-producing animals are beta-lactams, tetracyclines, aminoglycosides, quinolones, macrolides and sulfonamides. Oxytetracycline (OTC, belongs to tetracycline group of antimicrobials) is a broad spectrum antibiotics and commonly used in livestock and poultry production as a therapy as well as AGPs in Bangladesh. However, the use of this compound may result in residues in animal derived food products, especially if withdrawal times are not observed. These residues may pose a health threat to consumers, depending on the type of food and the amount of residue present. The acceptable maximum residue limit (MRL) for OTC as recommended by the joint FAO/WHO Expert Committee on Food Additives (1999) is 0.2, 0.6 and 1.2 ng/g for beef, liver and kidney, respectively. Human health problems resulting from intake of sub-chronic exposure levels of OTC include gastrointestinal disturbances (Baker and Leyland, 1983), teratogenic risk to the fetus, allergic reactions (Schenk and Collery, 1998) and development of resistant pathogens for human and animals (Van de Bogaard and Stobberingh, 2000). Besides tetracycline, sulfonamides are also generally used as therapy and AGPs in food producing animals in Bangladesh. 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.

Eid-ul-Adha is the second largest religious festival of Muslims in Bangladesh where huge number of cattle, buffalo and small ruminants sacrificed by Muslims in every year. These animals especially cattle are sold with good price and owners get good profit during the big festival. Therefore, farmers prefer the fattening programme of cattle on ahead of the big festival as one of the good source of income. To decrease the production cost AGPs are used in the feed of beef cattle without considering proper withdrawal period. Therefore, there are risks of antibiotic residue in the beef of cattle those are slaughtered during the big festival.

The availability of simple and reliable screening system for the detection of antibiotics is an essential tool in assuring the safety of food products. Modified four plate test and thin-layer chromatography can detect residues of (beta-lactam, tetracycline and sulfonamide antibiotics) but they did not allow quantification of these residues. However, high performance liquid chromatography (HPLC) can be a reliable method to quantify the drug residue present on slaughtered animal's product. Therefore, the research was carried out to detect and quantify the concentration of antibacterial residues using HPLC in raw beef of cattle slaughtered during Eid-ul-Adha.

Materials and Methods

Study design

A cross-sectional survey was carried out during Eid-ul-Adha in 2018 in Mymensingh district which is the third largest city and fourth-most populous urban agglomeration in Bangladesh. In order to obtain the representative samples a survey was conducted to the different communities who sacrificed beef cattle during the day of Eid-ul-Adha in 2018.

Sample size determination

Sample size required for the study were determined using this formula ($n=z^2pq/d^2$) where

z=1.96, p = antimicrobial prevalence=0.07, q=1-p=0.93 and d=0.05 (Taherdoost 2017).

Sample collection

A total of 100 raw cattle beef samples were collected from 4 Upazilas of Mymensingh district. Of them 25 samples were collected from each of 4 Upazilas (Gafargaon, Fulpur, Dhobaura, Bhaluka). Approximately 50g of beef samples were collected in beef collection zip bags from each animal and immediately cooled to 4°C in the cool box container and transported to the laboratory in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University for quantitative analysis of antibiotic residues. The samples were stored in freezer at -20^{0}C before analysis.

Selection of antibacterial drugs for analysis

During preliminary survey, it was observed that OTC and sulphadimidine (SDI) antimicrobial drugs were found most frequently in use at different livestock farms of Bangladesh. On the basis of surveyed observation, OTC and SDI were selected for quantification of residue in raw beef samples.

Estimation of drug residues in cattle beef through High Performance Liquid Chromatography (HPLC)

Preparation of standards antibiotic solutions sample

The stock antibiotic standards at the conc. of 1000µg/L of OTC and SDI were prepared individually in methanol (HPLC grade). The working solutions at the conc. range of 50–10000µg/L were made from the stock solutions of the individual antibiotics.

HPLC analysis

Residues of OTC and SDI were quantified in raw beef samples through HPLC based on the method described previously (Cinquina *et al*., 2003) with slight modification. Approximately 2.5-3.0g of grinding beef sample was transferred to the centrifuge tube and mixed with 5ml phosphate buffered saline. Then the sample was mixed with 1.5ml 30% TCA and centrifuged at 3000 rpm for 30 min to remove the fat. The supernatant was collected and mixed with 2ml of di ethyl ether and mixed for 10 min. Finally sufficient amount of sediment collected and 20µl of it was injected to the HPLC system. Preliminary, an approach was applied to detect/quantify OTC and SDI in single gradient solvent program (Table 1).

Table1. Gradient program applied for sulphadimidine and oxytetracycline

Time (min)	Flow rate (ml/min)	Acetonitrile (%)	Deionized water (%)
0	1	20	80
15	1	50	50
15.1	1	20	80
20	1	20	80

The injection volume was 20µl and the UV detection was performed at 270nm for all drugs.

Results

Quantitative analysis of OTC in beef samples

An overall 59 % (59/100) beef samples were found to be positive for OTC residue. The mean residual conc. (MRC) of OTC was 0.145µg/ml which ranged from 0.104 to 0.435µg/ml. Moreover, 19% samples contained residues above the MRL value. Among them 36% samples from Gafagaon upazila were found to contain OTC residue above the MRL value. In addition, 24% samples from Fulpur upazila were found to contain OTC residue above the MRL (Table 2).

Table 2. Level of oxytetracycline residues in raw beef samples

Location	No. of samples screened	Positive for OTC (%)	Conc. range (µg/ml)	Mean residual Conc. (µg/ml)	No. of samples above MRL (%)
Gafargaon	25	20 (80)	0.095-0.305	0.256	9 (36)
Fulpur	25	15 (60)	0.125-0.279	0.234	6 (24)
Dhobaura	25	10 (40)	0.078-0.216	0.099	2 (8)
Bhaluka	25	14 (56)	0.098-0.437	0.222	2 (8)

Total	100	59 (59)	0.104-0.435	0.145	19 (19)

Quantitative analysis of sulphadimidine in raw beef samples

An overall 43% (43/100) beef samples were found to be positive for sulphadimidine (SDI) residues. The MRC of SDI was 0.094µg/ml which ranged from 0.083 to 0.356µg/ml. However, 12% (12/100) samples contained residues above the MRL. The highest number of samples 16% (4/25) from Gafargaon upazila were found to contain SDI residue above MRL (Table 3).

Table 3. Level of sulphadimidine residues in raw cattle beef samples

Location	No. of samples screened	Positive for SDI (%)	Conc. range (µg/ml)	Mean residual Conc. (µg/ml)	No. of samples above MRL (%)
Gafargaon	25	12 (48)	0.087-0.140	0.089	4 (16)
Fulpur	25	9 (36)	0.084-0.189	0.135	3 (12)
Dhobaura	25	15 (60)	0.097-0.437	0.146	4 (16)
Bhaluka	25	7 (38)	0.084-0.287	0.094	1 (4)
Total	100	43 (43)	0.083-0.356	0.094	12 (12)

Discussion

The residues of two commonly used antibacterials like OTC and SDI in raw cattle beef were quantified using the HPLC coupled with UV detector (HPLC-UV). HPLC-UV technique is usually adopted as a confirmatory method for the antibiotic residues in animal based foods (Sorensen *et al.*, 1997; Taguchi *et al.* 1999; Faria-Reyes *et al.*, 2000). In this method a single gradient solvent program was developed and applied to detect and separate the two antimicrobial drugs (SDI and OTC). It is important to note that the single mobile gradient program for the separation and/or detection of SDI through HPLC is new and has not been reported before. No doubt, many studies reported single gradient solvent program in a similar fashion but these were for only multiple drugs of similar group/class of antibiotics. It was observed that farmers used antibiotics indiscriminately without the guidance of veterinarians. Farmers also did not follow withdrawal period and there is also no regulatory control of residue in foods of animal origin. Consequently, residual contamination in food of animal origin is common (Hakem *et al.*, 2013) that may pose significant threat to public health (McEwen and McNab, 1997). It was also noted that the use of antibiotics like OTC and SDI were abundant at the livestock/ farms in the vicinities of Mymensingh district and this is probably related to availability of these drugs in the markets, low cost and better curative performance (Gebre, 2012). OTC, belongs to tetracyclines, are broad-spectrum antibiotics widely used in animal husbandry for either prevention or treatment of diseases and AGPs in food-producing animals (Abbasi *et al.*, 2011).

An overall 59% and 43% raw beef samples contained OTC and SDI, respectively Ibrahim *et al.*, (2009) in reported that 8% of slaughtered cattle contained tetracycline residues. On the other hand, Mehtabuddin *et al.* (2012) stated that 43% beef samples were contaminated with sulfonamide residues. In another study, 71.3% beef samples had noticeable OTC residues, (Bedada, *et al.*, 2012). Reason behind the higher drug residual contamination in foods of animal origin might be attributed with unregulated and indiscriminate use and non-respect of withdrawal periods for slaughtered animals (Hakem *et al.*, 2013). Nevertheless, the detection of antibiotic residues in beef reveals the irrational use of the antimicrobial drugs by the livestock farmers, and/or withdrawal periods was not followed before slaughtering of animals. It is important to note that several factors like prescription of antimicrobial drugs, the administration route and subsequent residue depletion, the attitude towards withdrawal times, etc. might affect the level of residues in beef (Pikkemaat *et al.*, 2011). We observed that 19% and 12% beef samples contaminated with OTC and SDI residues above MRL, respectively.

Similar findings were also reported by other authors (Senyuva *et al,* 2000; Mangsi, 2014; Muriuki *et al.,* 2001; Myllyniemi, 2004). The presence of OTC and SDI in cattle beef indicates that antibiotics were used indiscriminately as a growth promoter in this study area during Eid Festival.

Conclusions

The results indicate that drug residue is common in raw cattle beef samples and a good proportion of beef samples exceeded MRL. Farmer's education to ensure rational use of antibiotics/antibacterial and to follow withdrawal period may help control this public health hazards in Bangladesh context. Moreover, regular monitoring of drug residues by food safety authority and reward and punishment where applicable might help decrease the residue problem.

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