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Estimation and Health Risk Assessment of Toxic Metals and Antibiotic Residues in Meats Served at Hospitals in Egypt

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Abstract

Food served to patients at hospitals should be free from chemical contaminants such as heavy metals and antibiotics. This study was taken to estimate the residual concentrations of lead (Pb), cadmium (Cd), copper (Cu) and arsenic (As) in raw and cooked beef and chicken served at Zagazig university hospitals, Egypt. Dietary intake and health risk assessment were calculated for the examined metals. Furthermore, antibiotic residues were screened in the collected samples using the four plate method. Oxytetracycline residues were further quantitativly estimated in the positive samples. The achieved results indicated contamination of the examined samples with metals in different percentages. The recorded concentrations were higher than the maximum permissible limits for Pb, Cd and As. Cooking did not significantly alter the load of the metals. Antibiotic residues were detected only in raw meat and chicken and disappeared after cooking. Strict control measures should be adopted to food served in hospitals.

Keywords: Chemical hazards; Heavy metals; Antibiotic residues; Oxytetracycline; Hospitals

Introduction

Meat is a major food served at hospitals worldwide due to its palatability and high nutritive value. Contamination of meat with xenobiotics such as heavy metals and antibiotics represents a great public health concern [1]. However, limited information is available about meat safety, in terms of chemical residues.

Chemical residues in meat served at hospitals may lead to sever toxicological implications especially among patients from children, simply because their weak immune systems and high susceptibility to adverse outcomes. Thus, estimation of health risks among adults and children in due to intake of such contaminants is of a significant value.

Heavy metals and trace elements find their way into human and animal bodies mainly through food intake. Metals can contaminate meat either during the lifetime of the animal or during post-slaughter processing via cooking utensils, raw materials, spices and food packaging [2]. Intake of heavy metals may lead to severe adverse effects. For instances, lead (Pb) is responsible for many deaths among children in China, Nigeria and Zambia [3-5]. High exposure of people to cadmium (Cd) leads to sever kidney and pulmonary dysfunction and impairment of bone development [6]. Arsenic (As) is usually added to animal feed formulas, however, high levels of As are responsible for dermatitis and neurological symptoms [7]. Copper (Cu) is a trace element that is needed in small concentrations for the normal enzyme functions in the body, however, high Cu levels are linked to kidney and liver dysfunction [8].

Antibiotics are used mainly for disease treatment and growth promotion, however, lack of awareness about drug's withdrawal times can result in drug residues in animal products causing many health hazards and appearance of drug-resistant microorganisms. Oxytetracycline is a broad-spectrum antibiotic that is frequently used for prevention and control of several bacterial diseases in many African countries [1]. However, there is limited information about antibiotic residues in meat served at hospitals. Therefore, the objectives of this study were firstly to estimate Pb, Cd, Cu and As in meat served at hospitals including beef and chicken meat either raw or cooked. Secondly, estimation of dietary metal intake and health risks associated with consumption of contaminated meat was conducted. Thirdly, screening of antibiotic residues in such food stuffs were also conducted. Public health significance of such food-contaminants was also discussed.

Materials and Methods

All experiments were done according to guidelines of Zagazig university, Egypt. All chemicals were HPLC grade or the highest quality available and purchased from Merk, Darmastadt, Germany.

Collection of samples

A total of one hundred samples were collected randomly and equally from raw meat, raw chicken, cooked meat and cooked chicken (25 each) from Zagazig university hospital kitchens, Zagazig, Egypt (Figure 1) during the period of February to October 2017. Each sample was transferred cooled to the laboratory and kept at -20°C till time of heavy metal and antibiotic residues measurements.
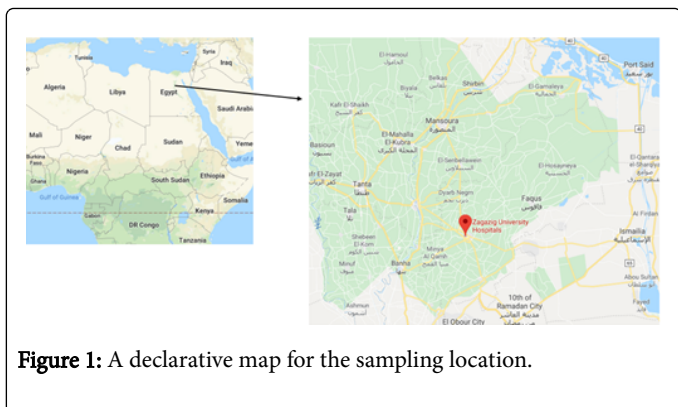
Figure 1: A declarative map for the sampling location.

Heavy metal measurement and risk analysis

Sample preparation: one gram of each sample was digested in 3 ml nitric acid 65% and 2 ml perchloric acid 70% [9]. The content was left to stand overnight at room temperature in falcon tubes. Then, these tubes were incubated at 70°C for 3 hours in water bath with swirling at 30 min intervals during the heating period. Tubes were left to cool at room temperature, diluted with 20 ml de-ionized water, and filtered by using filter paper. The filtrate was kept at room temperature until analysis for heavy metal contents.

Analytical procedures: Levels of As were measured using hydride generation/cold vapor atomic absorption spectroscopy; while graphite furnace was used in case of Pb, Cd and Cu (Perkin Elmer® PinAAcle™ 900 T atomic absorption spectrophotometer (Shelton, CT, USA).

Quality assurance and control measures: The reference material; DORM-3 (Fish protein, the National Research Council, Canada) was used to ensure the accuracy and validity of the analytical procedures of heavy metals. Recovery rates ranged from 90% to 115%. All analyses were done in triplicates including those of blanks and standards. Residual concentrations of Pb, Cd, As and Cu were expressed as µg/g wet weight (ppm).

Dietary intake and human health risk assessment: The estimated daily intake of heavy metals (EDI) (µg/kg/day) was calculated by the following equation:

$$EDI = Cm * FIR / BW \text{ [10]}$$

Where Cm is concentration of metal; FIR is the meat ingestion rate, 85.7 g/day for red meat [11] and 100 g/day for chicken meat [12]; BW is the average body weight, 20.5 for child and 60.7 kg for adult [13].

Health risks were assessed by evaluation of carcinogenic and non-carcinogenic effects of heavy metals [10]. Non-carcinogenic risks were evaluated through the following equation:

$$HR = EDI / RFD * 10^{-3}$$

where HR is hazard ratio; EDI is the estimated daily intake; RFD is the oral reference dose (4E-3, 1E-3, 3E-4 and 4E-2 mg/kg/day for Pb, Cd, As and Cu, respectively [10]). Hazard index (HI) was calculated as $HI = \sum HRi$ where; i represents each metal, HI>1 indicates potential risk; while HI ≤ 1 indicates no risk.

Carcinogenic risks were evaluated by detection of incremental lifetime cancer risk (ILCR), where $ILCR = CSF * CDI$

where CSF is the cancer slope factor (CSF values were set as 1.5, 6.3 and 0.0085 (mg/kg/day) for As, Cd and Pb respectively [10]). CDI is the chronic daily intake of metals mg/kg/day and it was calculated based on the following equation: $CDI = EDI * Efr * Edtot / AT$; where Efr is the exposure frequency (365 days/year); Edtot is the exposure duration 70 years and AT is the period of exposure for carcinogenic effects (70 years life time) [14]. ILCR is acceptable within the range of 1×10^{-6}-1×10^{-4} [15].

Antibiotic residues measurements

Screening of antibiotic residues using microbial inhibition assay: In order to screen the incidence of antibiotic residues in the the examined samples, microbial inhibition assay was conducted according to the method of Koenen-Dierick et al. [16]. *Bacillus subtilis* BGA strain was used as the test organism. Mueller-Hinton agar plates were prepared at pH 7.0. A sterile 8 mm diameter cork borer was used to make wells in the agar medium for introduction of meat samples. Meat samples were introduced into wells made in the Mueller-Hinton agar medium. Then agar plates were incubated at 37°C for 24 h. After incubation, a zone of inhibition of 1 cm or more was considered a positive case of meat sample containing drug residues.

Quantitative analysis of oxytetracycline residues in meat samples: The sample extraction, detection and quantitation was carried out according to the method of Jevinova et al. [17]. In brief, a mixture consists of 2 ml of meat homogenate, 0.1 ml citric acid, 1 ml nitric acid (30%), 4 ml methanol and 1 ml of deionized water was prepared and ultrasonicated for 15 min and centrifuged at 5300 rpm for 10 min. After filtration (0.45 µm nylon filter), a 20 µl of solution was injected into HPLC system (A constant liquid chromatography pump provided with an autosampler plus surveyor, ThermoScientific Company, USA) for analysis.

Chromatographic condition: A mobile phase of methanol and formic acid 0.1% using a gradient method with a flow rate of 1.5 ml/min at 25°C was used. The separation was done on hypersil gold C18 (10 µm, 100 × 4.6 mm) column with mobile phase. Detection was performed with PDA detector set at 350 nm wave length. Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the software (Chromo Quest 5).

Calibration curve: Calibration curve was prepared using concentrations of 0.01, 0.1, 0.5, 1.25, 2.5, 5, 10, 20, 50 mg/L of oxytetracycline in eluent. The detection limit was 0.01 ppm while the retention time was 3.7 min for oxytetracycline.

Statistical Analysis

Statistical significance was evaluated using Tukey–Kramer honestly significant difference tests, with p<0.05 considered as significant (JMP program, SAS Institute, Cary, NC, USA).

Results and Discussion

Investigation of chemical residues and their related health hazards in meat served at hospitals had received less attention. In this study, heavy metal and antibiotic residues were estimated in meat served at Zagazig university hospitals, Egypt.

Levels of heavy metals in meat and chicken meat samples

Meat is an important source of protein, fat and minerals. Content of heavy metals in meat served at hospitals should be minimum to avoid

interactions with medications and the metal-related adverse effects. In Zagazig university hospitals, meat sources include either beef or chicken. The results recorded in Table 1 showed that all examined samples were contaminated with Pb, Cd, As and Cu. There was no significant difference in metal content among beef and chicken samples. The residual concentrations of metals recorded in raw beef and chicken meat in this study go partially in agreement with levels recorded in our previous report and to levels recorded in Bangladesh [9,18]. However, lower concentrations were recorded in Belgium and Ghana [14,19], and higher concentrations were recorded in Pakistan [20]. European Food Safety Authority (EFSA) had set maximum residual concentrations (MPL) for Pb (0.1 ppm), Cd (0.05 ppm) and as

(0.01 ppm) [21]; in this study, 66.7%, 58.8% and 80% of the examined samples exceeded MPL of Pb, Cd and As, respectively. None of the examined samples exceeded MPL of Cu (40 ppm) set by Codex Alimentarius Commission [22]. It notes worthy to mention that most of studies performed on estimation of heavy metal residues in food subjects were done on raw samples, although most of foods are consumed cooked. Interestingly, cooking led to a clear elevation in the content of the toxic metals which was significant in case of cooked chicken meat (Table 1). This observation may be attributed to the water evaporation and water loss leading to concentration of the metals in the cooked tissue [23].

Samples	Pb		Cd		Cu		As	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Raw beef	0.041-0.393	0.245 ± 0.069[a]	0.011-0.175	0.089 ± 0.042[a]	1.043-3.476	2.244 ± 0.229[ab]	0.008-0.043	0.022 ± 0.011[a]
Raw chicken	0.062-0.457	0.248 ± 0.073[a]	0.019-0.217	0.114 ± 0.054[a]	0.771-2.191	1.629 ± 0.166[a]	0.015-0.124	0.068 ± 0.029[b]
Cooked beef	0.072-0.519	0.298 ± 0.081[a]	0.022-0.256	0.115 ± 0.055[a]	1.279-3.551	2.354 ± 0.231[b]	0.012-0.057	0.029 ± 0.0094[a]
Cooked chicken	0.111-0.754	0.421 ± 0.127[b]	0.045-0.393	0.202 ± 0.061[b]	0.825-2.645	1.598 ± 0.233[a]	0.026-0.178	0.122 ± 0.048[c]

Table 1: Elemental concentrations in the examined meat samples (mg/ kg). Columns that carry different superscript letter are significantly different at P<0.05.

Estimated daily intake of heavy metals and health risk assessment

Exposure of humans to heavy metals is associated with many adverse health effects and toxicological implications, especially if patients were among the exposed groups. One major task of food hygienists is to ensure safety of foods served to people. Thus dietary exposure and risk assessment due to the ingestion of metal-contaminated meat were evaluated. The EDI values ranged between 0.346 and 2.054 μg/kg/day for Pb, 0.126 and 0.985 μg/kg/day for Cd, 2.633 and 9.84 μg/kg/day for Cu and 0.031 and 0.585 μg/kg/day for as (Table 2). The EDI values for Pb, Cd, Cu and as were lower than the established tolerable daily intake in all samples (established TDI values (μg/kg/day) were 3.57 (Pb), 1 (Cd), 500 (Cu), 2.1 (As) [14]).

Samples	Age	EDI				HR				HI
		Pb	Cd	Cu	As	Pb	Cd	Cu	As	
Raw meat	Adults	0.346	0.126	3.168	0.031	0.087	0.126	0.079	0.103	0.4
	children	1.024	0.372	9.381	0.092	0.256	0.372	0.235	0.307	1.2
Raw chicken	Adults	0.409	0.188	2.684	0.112	0.102	0.188	0.067	0.373	0.7
	children	1.211	0.556	7.946	0.332	0.303	0.556	0.199	1.107	2.2
Cooked meat	Adults	0.421	0.162	3.324	0.041	0.105	0.162	0.083	0.137	0.5
	children	1.246	0.481	9.84	0.121	0.312	0.481	0.246	0.403	1.4
Cooked chicken	Adults	0.694	0.333	2.633	0.198	0.174	0.333	0.066	0.661	1.2
	children	2.054	0.985	7.795	0.585	0.514	0.985	0.195	1.951	3.6

Table 2: EDI (μg/kg/day), HR, and HIs of Pb, Cd, Cu and As. Values highlighted in bold are higher than 1.

The HR values of Pb, Cd and Cu did not exceed one in case of both adults and children. However, children had HR values of 1.107 and 1.951 for As in case of raw and cooked chicken meat. Additionally, children were at potential risk HI>1 for both cooked meat and chicken (Table 2). Incremental lifetime cancer risks were unacceptable especially for Cd and as among children group (Table 3). Nearly similar values were reported by Darwish et al. [9]. However, lower figures were reported by Bortey-Sam et al. [14]. International Agency for Research on Cancer categorized As, Cd and Pb as carcinogenic metals [24], the highest cancer risk was calculated as 9.1×10^{-3} this indicates that cooked chicken would result in 91 cancer cases per 10000 people.

Samples	Age	Pb	Cd	As	∑ILCR
Raw meat	Adults	2.9×10^{-6}	1.3×10^{-4}	4.7×10^{-5}	1.4×10^{-4}
	children	8.7×10^{-6}	2.3×10^{-3}	1.4×10^{-4}	2.4×10^{-3}
Raw chicken	Adults	3.5×10^{-6}	1.2×10^{-3}	5.6×10^{-4}	1.8×10^{-3}
	children	1×10^{-5}	3.5×10^{-3}	1.7×10^{-3}	5.2×10^{-3}
Cooked meat	Adults	3.6×10^{-6}	1×10^{-3}	6.2×10^{-5}	1.1×10^{-3}
	children	1.1×10^{-5}	3×10^{-3}	1.8×10^{-4}	3.2×10^{-3}
Cooked chicken	Adults	5.9×10^{-6}	2.1×10^{-3}	9.9×10^{-4}	3.1×10^{-3}
	children	1.7×10^{-5}	6.2×10^{-3}	2.9×10^{-3}	9.1×10^{-3}

Table 3: Incremental lifetime cancer risks of Pb, Cd and as on adults and children health.

The possible toxicological implications and adverse effects of lead among patients may include hemolytic anemia, atherosclerosis, liver apoptosis, atrophy of the ovary and renal toxicity [25]. Lead also has a specific affinity for bones leading to osteoporosis and interferes with iron and manganese metabolism; and associated with pathological changes in organs and the central nervous system, leading to decrements in intelligence quotients in children [18]. Cadmium chronic intake may lead to severe respiratory symptoms, nephrotoxicity, glucosuria, aminoaciduria and decrease in the glomerular filtration rate, hypertension, hepatic injury and lung damage. Furthermore, cadmium causes osteoporosis, osteomalacia and Itai-Itai disease [6]. Copper toxicity can cause anemia, brain, liver, kidney damage, stomach and intestinal irritation. Additionally, Cu can lead to Menke's and Wilson's diseases [26]. Chronic arsenic exposure causes inflammatory, degenerative and neoplastic changes of cardiovascular, nervous, reproductive, respiratory and haematopoetic system [27].

Antibiotic residues in meat and chicken samples

Exposure of human to antibiotic residues via meat-intake constitutes a major health hazards via development of antibiotic resistant bacterial strains in addition to some allergic reactions, which may develop anaphylactic shock [1].

Samples	Inhibition zone (mm)		Oxytetracycline concentration (µg/g)	
	N	Mean ± SE	N	Mean ± SE
Raw meat	4	3.75 ± 0.51	1	0.178
Raw chicken	10	4.31 ± 0.31	4	0.292 ± 0.152
Cooked meat	0	0	0	0
Cooked chicken	0	0	0	0

Table 4: Antibiotic residues in the examined meat samples. *N: Number of positive samples.

The microbial inhibition assay revealed that 4 (16%) and 10 (40%) of the examined raw meat and raw chicken were contaminated by antibiotics, inhibition zone diameters were 3.75 ± 0.51 and 4.31 ± 0.31 mm (Table 4). As oxytetracycline is the major antibiotic used in Egypt

in chicken and livestock disease prevention and control, the residual concentrations of oxytetracycline were estimated using HPLC. One meat sample had detectable concentrations of oxytetracycline (0.178 µg/g). Unlikely; oxytetracycline could be detected in four raw chicken meat samples. The average concentration of the tested antibiotic was 0.292 ± 0.152 µg/g (Table 4). Interestingly, antibiotics disappeared in the cooked meat samples; this may be attributed to the destructive effects of heat on antibiotics. The results of this study were in agreement with Morshdy et al. [28]; but Kimera et al. [29] recorded higher values in cattle meat marketed in Tanzania.

Conclusion

In conclusion, the results of this study declared that meat served at Zagazig university hospitals, Egypt is a potential source of human exposure to heavy metals and antibiotics and might constitute health hazards especially for the resident patients. Thus, strict control measures and legislations should be taken by the responsible authorities to reduce such hazards.

Acknowledgments

Conflict of Interest

None.

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