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The Potential Impact of Rigor Mortis on Antibiotic Residues in Bovine Ribeye and Kidney

Eslam Arafa[1], Shimaa Edris[1], Omar Ahmed-Farid[2] and Islam Sabike[1]*

[1] *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Toukh, Qalyubia 13736, Egypt, eslam.Arafa21@fvtm.bu.edu.eg, Shimaa.edrees@fvtm.bu.edu.eg, islam.sabek@fvtm.bu.edu.eg.*

[2] *Egyptian Drug Authority (EDA, formerly: NODCAR), Giza, Egypt, https://orcid.org/0000-0002-1020-5777, ebntaimya@yahoo.com.*

Abstract

THE CURRENT investigation aimed to evaluate the effects of rigor-mortis durations, ranging from four to forty-eight hours, on antibiotic residues found in bovine ribeye and kidney samples. The study's hypothesis is that some rigor mortis cascades can change and even destroy structural meat components, which affects the quality of the meat. Thirty random samples of fresh ribeye loin (Musculus longissimus thoracis et lumborum) cut and kidney (15 of each) were obtained from various butchers in Cairo, Egypt, and promptly delivered to the laboratory for analysis. The mean pH48h results of the ribeye samples were substantially lower than the mean pH4h. A 48-hour rigor-mortis with chilling at 4 °C shows statistically significant reducing effects on concentrations of 41.6% (5/12) of antibiotic residues detected in ribeye, including amoxicillin, ampicillin, ciprofloxacin, norfloxacin, oxytetracycline, and tylosin ($P < 0.05$). In contrast, antibiotic-residues in the kidney were not affected by the 48-hour postmortem duration. The antibiotic-residues declining curve could be caused by postmortem meat fluid loss, such as purge loss, and the potential occurrence of antibiotic residue biotransformation under rigor-mortem pathways. Nonetheless, the current study limitations should be the focus of future research involving many antibiotic-positive samples, focusing on discovering reaction products that may be generated postmortem from proposed postmortem biotransformation cascades such as hydrolytic cleavage of different antibiotic residues. Finally, the investigations on animals using known antibiotics should be undertaken to validate and/or rule out this possibility.

Keywords: antibiotic residues, Egyptian butcheries, pH48h, ribeye meat and kidney, rigor-mortis.

Introduction

Antibiotics are among the most important veterinary medicine substances connected with animal feed and food animal production. From basic production to final consumption, there are several routes in the food supply chain where food may be contaminated with chemicals, including antibiotics [1]. Animals are administered veterinary medications through injection, oral administration, or animal feed. These are the same means by which animal-derived food is exposed to antibiotic residue. Most of the time, the medications are mixed with the food [2]. The use of veterinary medications in food-producing animals may result in the presence of parent compounds and/or metabolite residues in food items, which can be detrimental to humans. One of the main ways that humans are exposed to these undesirable compounds is through food, either from plants or animals [3].

Antibiotics were banned as growth promoters for many years. Their use in animals may leave residues in food, which could be harmful to consumers' health as well as the environment, technology, and welfare of animals. Antibiotic residues have the potential to produce and exacerbate severe diseases, including anaphylactic shock, nephropathy (gentamicin), bone marrow toxicity, mutagenesis effects, and reproductive abnormalities (chloramphenicol). Antibiotics, including β-lactams, tetracyclines, fluoroquinolones, sulfonamides, and macrolides, are widely used in veterinary medicine [4].

The existence of veterinary antibiotic residues in animal products such as meat may also induce allergies (penicillin) and cause cancer

*Corresponding author: Islam Sabike, E-mail: islam.sabek@fvtm.bu.edu.eg, Tel.: 01027463990
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(sulfamethazine, oxytetracycline, and furazolidone) [5, 6]. One of the most serious health problems is the development and spread of antibiotic resistance across the food chain [7-9]. All individuals are vulnerable, but some are more vulnerable than others. These include the elderly, immunocompromised, pregnant women, new-borns, small children, and others, whose numbers are projected to rise [10].

Antimicrobial use, quantities and usage patterns, monitoring (AMU) is a valuable data source that may be utilized in conjunction with antimicrobial resistance (AMR) surveillance to evaluate and control AMR-related hazards [11]. Antibiotic residues are typically detected using a variety of traditional analysis techniques, but the most recent approach, liquid chromatography-tandem mass spectrometry (LC-MS/MS), has excellent sensitivity, selectivity, and accuracy when compared to other methods like gas chromatography (GC), capillary electrophoresis (CE), enzyme-linked immunosorbent assay (ELISA), and liquid chromatography (LC) [5]. Although the LC-MS/MS screening approach necessitates time-consuming processes and costly equipment, the accuracy of the data renders it trustworthy and necessary for use in research investigating scenarios and/or factors that could influence residue levels.

Rigor mortis is a complex biochemical process in the conversion of muscle to meat that happens relatively early postmortem (PM) and is marked by meat hardness. Despite oxygen deprivation in early postmortem (PM), the muscles continue to function and engage in anaerobic metabolic activity to produce high-energy phosphate molecules (adenosine triphosphate, ATP) from glycogen, allowing for muscular relaxation. However, due to a lack of an effective antioxidant system, lactate and hydrogen ions (H+) from glycolysis and ATP hydrolysis accumulated, causing a pH reduction [12, 13].

This complex biochemical process is governed by various proteins that work together to control a variety of meat quality traits. These proteins include metabolic enzymes, heat shock proteins (HSPs), oxidative proteins, structural proteins, and proteases [12, 13]. Proteases such as calpains, caspases, lysosomal cathepsins, and proteasomes hydrolyze structural proteins to make meat softer. Also, postmortem meat color and pH are influenced by metabolic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and glycogen phosphorylase (PYGM). Meat quality can be regulated by heat shock proteins (HSPs) such as HSPB1, αβ-crystallin, HSP40, HSP70-8, and HSP90 [14]. Key antioxidant players in the peroxide cleavage scavenging include peroxiredoxins (1, 2 and 6), and superoxide dismutase (SOD) [15]. Other postmortem enzymes with subsequent effects on the pH and *a** value of meat include beta-enolase (ENO3), phosphoglucomutase-1 (PGM1), protein-L-isoaspartate O-methyltransferase (PCMT1), and proteasome subunit beta type-2 (PSMB2) [16].

These postmortem metabolic cascades are reported to have sufficient power to regulate a variety of meat quality factors [12,13]. Moreover, in the past, meat has been shown to degrade penicillin and ampicillin, and the degradation in bovine tissue is highly varied and appears to be depending on the cooking temperature and duration [17]. The author is unaware of any conclusive or effective studies on the effect of degradation of this class of antibiotics in food [18]. Thus, the hypothesis of this study relies on the probability that antibiotic residues may be impacted by the progression of various post-mortem cascades, particularly proteases, and metabolic and antioxidant enzymes that impact meat structural quality, such as tenderness. In veterinary medicine, monitoring antibiotic residues in kidney or meat samples is essential to guaranteeing food safety and regulatory compliance. The goal of residue detection is to stop antibiotic residues from endangering human health by preventing them from getting into the food chain. Therefore, to shed light on the possible influence of rigor-mortem on antibiotic-residues and provide a potential preventive and control measure for related public health hazards, the current study compared antibiotic residues immediately after slaughtering and 48 h of rigor mortem progression in meat cuts and kidneys randomly purchased from Egyptian butcheries.

Material and Methods

Experiment management and approval.

All methods used in this study were approved by the Benha University, Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee Research Ethics number (BUFVTM 24).

Sample collection

Thirty random samples of fresh ribeye loin (*Musculus longissimus thoracis* et *lumborum*, *LTL*) cut and kidney (15 of each) were obtained from multiple butchers in Cairo, Egypt, and immediately sent to the laboratory for analysis.

Sample preparation and distribution.

All samples originated from male cattle of various ages. Each fresh ribeye loin (*Musculus longissimus thoracis* et *lumborum*, *LTL*) was aseptically prepared from both sides of the carcass and weighed approximately 500 g. To prevent meat

shortening and ensure rigor mortis progression, samples were kept at room temperature 25 ºC for 4 h in a Binder KB chilling incubator (BINDER GmbH, Tuttlingen, Germany) before being tested for pH4h. After measuring the pH4h, two samples weighing about 100 g (50 each) were dissected and frozen at -21 ºc to evaluate antibiotic residual levels. The remaining sections were chilled for 48 hours at 4 ± 0.5 °C in a Binder KB chilling incubator (BINDER GmbH, Tuttlingen, Germany) before being retested for pH_{48h} and frozen to estimate changes in antibiotic residue levels after the rigor mortis phase.

pH analysis

After the designated chilling time, samples were left at room temperature for 30 min before the *LTL* pH_{48h} was applied. The pH was ascertained by subjecting the *LTL* pH_{48h} samples directly to pH-meter electrodes (Jenway 3510 pH-meter, Cole-Parmer, Staffordshire, United Kingdom). Three different pH levels (10, 4, and 7) were used to calibrate the pH meter at room temperature in conjunction with a temperature metal probe [19].

Liquid chromatography-tandem mass spectrometry analytical method (LC-MS/MS) [20]

Sample Preparation and residue extraction

Approximately 10 g of beef *LTL* muscle tissue was homogenized with a high-speed homogenizer. Then, 2 g of homogenized sample was transferred to a 50-mL centrifuge tube. Next, 10 mL of extraction solvent (80:20 v/v acetonitrile + 0.1% formic acid) was added. Vortex for 5 minutes, then sonicate for 10 minutes. Centrifuge at 4000 rpm for 10 minutes at 4 °C. The supernatant was transferred to a clean tube. 5 mL of hexane was added, vortexed for 2 minutes, and then centrifuged at 4000 rpm for 5 minutes. The overlying hexane layer was then discarded. The aqueous layer was passed through a solid-phase extraction (SPE) cartridge preconditioned with methanol and water. Five mL of methanol were used to elute the antibiotics, and the resulting eluates were evaporated to dryness at 40 °C under a nitrogen stream. 1 mL of mobile phase (50:50 v/v acetonitrile with 0.1% formic acid) was used to reconstitute the residue. After that, the reconstituted solution was filtered into an LC vial using a 0.22 µm syringe filter.

LC-MS/MS Analysis [20]

An LC-MS/MS system with an AB Sciex Triple Quad™ 5500 mass spectrometer and an Agilent 1260 Infinity HPLC system was used. Agilent ZORBAX Eclipse Plus C18 chromatography column with 2.1 x 100 mm and 1.8 µm particle size was installed. Two injectable mobile phases were added: acetonitrile with 0.1% formic acid (A) and water with 0.1% formic acid (B). The Gradient Program is set to begin with 90% A and 10% B, then progress linearly to 10% A and 90% B over 10 minutes, hold for 2 minutes, and return to initial conditions over 2 minutes. The flow rate was set to 0.3 mL/min, and the injection volume was 10 µL. The Mass Spectrometry conditions were Electrospray Ionization (ESI) in positive mode, Capillary Voltage of 4500 V, Source Temperature of 350 °C, Desolvation Temperature of 500 °C, and Multiple Reaction Monitoring (MRM) for specific antibiotic transitions.

Statistics

The data was analyzed using SPSS Version 22 (SPSS Inc., Chicago, IL, USA). The one-way ANOVA was used to investigate the effects of rigor mortis periods (48 h. for meat and kidney) on antibiotic residual levels in meat or kidney, where rigor mortis periods were considered fixed effects and ribeye meat, or kidney samples were considered random. The results are presented as means and standard errors. The statistical model used Tukey's b multiple comparison tests to compare antibiotic residual means. Significant differences were determined at a p value of <0.05.

Results

The pH values of ribeye samples taken 10 and 48 h after death are shown in Table 1, which is intended to evaluate the impact of the length of rigor mortis on antibiotic residues. The mean pH_{4h} values determined at four hours postmortem demonstrate that meat samples underwent the course of rigor mortis. The lowering trend apparent in all descriptive values (minimum, maximum, and mean) of pH_{48h} of ribeye samples reflects this pattern. Furthermore, in terms of statistical significance, the mean pH_{48h} of ribeye samples was lower than pH_4 ($P < 0.05$).

Table 2. Compare 4- and 48-hour postmortem different antibiotic residues concentrations in ribeye meat cut n = 15 collected from Egyptian markets. Only 37.5% (12/32) of the tested antibiotic residues were identified in the ribeye sample. In ribeye samples, there was statistically significant variation between the 4- and 48-hour postmortem concentrations of five antibiotic residues, namely amoxicillin, ampicillin, ciprofloxacin, norfloxacin, and tylosin, which were positively quantified using LC-MS/MS ($p < 0.05$). While slight reduction trend was noticed between the 4- and 48-hour postmortem concentrations of enrofloxacin, oxytetracycline, and gentamycin residues ($p > 0.05$).

Table 3 compares the amounts of various antibiotic residues in kidney samples (n = 15) obtained from Egyptian markets at four- and 48 h

postmortem periods. Of the monitored antibiotic residues, only 18.75% were found in kidney samples. The quantities of antibiotic residues positively identified using LC-MS/MS in kidney samples at 4 and 48 hours postmortem did not alter significantly, in line with ribeye samples ($p > 0.05$).

The amounts of various antibiotic residues positively detected in both chilled ribeye meat and kidney samples ($n = 30$) at four- and forty-eight-hours postmortem periods are displayed in Table 4. The results of a one-way ANOVA statistical analysis on thirty samples of chilled ribeye meat and kidney samples ($n = 30$) showed that rigor mortis for 48 hours significantly decreased the amount of Amoxicillin residue ($p < 0.05$). However, the remaining five residues—ampicillin, cefotaxime, ciprofloxacin, sulfonamides and gentamycin—shared by ribeye meat and kidney samples did not differ ($p > 0.05$).

Discussion

The objective of the current study was to assess the impact of rigor mortis, from 4- and 48-h, on antibiotic residues present in bovine ribeye and kidney samples obtained from Egyptian markets. The study's premise is based on the powerful capacity of some rigor mortis cascades to alter and even degrade structural meat components, which has an impact on meat quality.

There have been no previously published explanations for the impact of rigor mortis cascades on antibiotic residues, and this study may be the first to investigate this notion. The majority of the explanation for the findings, however, would involve correlating postmortem configurations and factors impacting antibiotic effectiveness in living animals to potential changes in antibiotic residues.

The pH curve obtained from postmortem meat is a reliable indicator of rigor mortis progression [21, 22]. The considerable falling trend seen in all descriptive values (minimum, maximum, and mean) of pH_{48h} of ribeye samples compared to mean pH4h indicates that ribeye meat samples underwent the rigor mortis process. Currently, recorded pH4h scores are in line with those previously recorded at the same range of temperature, but pH48h was higher [21,22]. In general, it is very difficult to suppress the formation of rigor mortis since the time for muscles to enter rigor mortis is short and the physiological and biochemical changes following slaughter are uncontrollable, but they could be slowed by cooling [23]. According to earlier research, the endogenous enzyme systems in bovine *M. longissimus dorsi* (*LTL*) muscles are at their peak at this pH range of 5.9–6.1 at 3 h post-mortem, thus often resulting in tender beef [22].

Prior research on the impact of preservatives and food processing techniques revealed that freezing is among the least successful methods for getting rid of antibiotic residues. On the other hand, using appropriate cooking techniques, such as multiple heat applications like frying and cooking combined, can help lower the antibiotic residue amount in food products [24]. Previously, meat has been demonstrated to degrade penicillin. According to a previous study, meat disintegrated ampicillin and penicillin, the rate of degradation in bovine tissue varied greatly and seemed to rely on the cooking temperature and time [17]. According to the current study, a 48-h rigor-mortis with chilling at 4 °C statistically alters or lowers five antibiotic residues: ampicillin, ciprofloxacin, norfloxacin, tylosin, and amoxicillin. Nonetheless, it is critical to emphasize the strong relationship between rigor-mortem cascades and tested tissue, such as the kidney or muscle, which ideally occurs in muscular tissue. In other words, the kidney does not exhibit the rigor mortis state that is typical of this type of muscle. The kidney is a parenchymatous organ that lacks muscle fiber and with approximately half the shelf-life of meat [25]. This may explain insignificant reduction occurred in all 6 detected antibiotic- residues in kidney tissue between 4- and 48-hour postmortem durations, which was also reflected in statistics when computed on the total muscle and kidney samples ($n= 30$).

Nonetheless, it is necessary to clarify two conclusions. The primary reason behind a declining trend in antibiotic residues was noticed between four- and forty-eight hours post-mortem. The second one is why this antibiotic residues reducing trend was not as significant as occurred in amoxicillin.

Current residue frequencies were examined to discover the variables contributing to the differential decline impact of 48 hours post-mortem on all screened residues in the current study residue. The authors argued that higher incidence rates of antibiotic residues, as those observed with amoxicillin, could result in representative variation and trustworthy statistical results. To put it simply, there were not enough or an equal number of positive samples for each antibiotic residue for statistical analysis, which could have an impact on the hypothesis. Another factor is that the positive antibiotic residue samples were gathered during a survey of various sources and had varying levels of residue. Approximately 67, 53, 37, 33, and 27% of LLM samples were positive for amoxicillin, ampicillin, ciprofloxacin, norfloxacin, and Tylosin, respectively. The other residue percentages in LLM were either 40, 27 or 17 or 23 or 50 or 50 or 13%, implying that the 48-h post-mortem declining impact

on current screened residues may be specific to an antibiotic class rather than study design factors such as the number and frequency of residue positive samples. Nonetheless, this encourages the research team to conduct additional investigations using a high number of positive samples to rule out such a possibility. Thus, future experiments on animals administered specific antibiotics and concentrations followed by rigor-mortem would be more accurate in determining rigor-mortem influence.

Regarding the causes of the observed declining trend in five antibiotic residues between four and 48-h post-mortem, the scenarios involved in the rigor-mortem after food animal slaughter could have played a significant role. However, the biotransformation of antibiotics in living animals should be discussed to highlight the prospect that comparable events may occur postmortem and contribute to the residues descending curve with prolonged rigor mortem. Postmortem meat fluid exudation, including purge loss due to a decrease in water holding capacity as a natural outcome of postmortem acidity and oxidative-induced lower proteolytic degradation of calpains, is most likely the contributing factor to the decreasing pattern in antibiotic residue between 4 h and 48 h postmortem. As rigor evolves, the intra-myofibril room for water shrinks, forcing entrapped (immobilized) water into the extra-myofibrillar gaps, where it is more easily lost as drip [26]. Thus, it should be noted that the postmortem antibiotic residues reduction pattern in meat would varies depending on the antibiotic's final distribution site, which can include interstitial fluid (extramyofibrillar gaps), cells, and within cells the various subcellular organelles [27]. To explain, non-lipophilic medicines, such as beta-lactam antibiotics, do not penetrate cells and are limited to the extracellular fluid volume [28]. Thus, more interstitial antibiotics, like amoxicillin and aminoglycosides, would exude as postmortem purge loss, which begins with free interstitial muscle water, than from those targeting cells (such as fluoroquinolones or macrolides) and various subcellular organelles. The primary classes of antibiotics exhibit bactericidal effects, which can be partially attributed to the induction of bacterial death through the massive production of reactive oxygen species (ROS) in tissues that are rich in mitochondria. This includes skeletal muscle, where ROS levels can be detrimental and affect the electron transport chain (ETC) and the tricarboxylic acid cycle (TCA) in mitochondria [29]. These circumstances deplete antioxidant capacity and allow reactive species to change the structure of skeletal muscle proteins, lipids, or nucleic acids and function, such as WHC [26], which results in increased exudation. These could imply that the degree of oxidation depends on the residue levels in the targeted region, and therefore exudation would vary. In muscle tissue, oxidizing circumstances appear to reversibly suppress calpain [30, 31]. Early postmortem proteolysis of intermediate filament proteins by μ-calpain and m-calpain can reduce water flow from within the cell to drip channels by preserving intramyofibrillar gaps. Reduced breakdown of proteins like desmin causes muscle cell shrinkage, which eventually leads to drip loss. In addition to pH and ionic strength, oxidative modifications can alter the structure of substrate proteins, reducing their susceptibility to μ-calpain breakage [30, 31]. In contrast, earlier studies indicated that tender meat categories exhibit higher levels of oxidative damage than intermediate and tough meat groups [15].

Regarding the present study hypothesis on the postmortem degradation of antibiotic residues, it should be mentioned that, in living animals, the antibiotic may be broken down by the body's metabolizing enzymes after it has been absorbed and dispersed throughout the tissues and tissue fluid. The liver is the principal organ of metabolism, but any biological tissue can metabolize drugs. The medication undergoes biotransformation or metabolic pathways, which convert it into either inactive, easily removed compounds or active, or highly active, metabolites [32]. There are two phases to the metabolism of many medicines. Phase I reactions are nonsynthetic and include the production of a new or changed functional group or cleavage (oxidation, reduction, hydrolysis). Phase II reactions are synthetic and entail conjugation with an endogenous material (such as glucuronic acid, sulfate, or glycine). Phase numbers indicate functional rather than sequential classification because certain medications only go through phase I or phase II responses [33]. In live animals, phase I biodegradation of xenbiotics occurs mostly by oxidation, which is mediated by the cytochrome P450 system, which is found solely inside the endoplasmic reticulum of hepatocytes and remains active for up to 72 hours after death [34]. During skeletal muscle ischemia, cytochrome c oxidase (COX) is activated to regulate mitochondrial respiration and apoptosis. Furthermore, Cyt*c* degrades reactive oxygen species (ROS). This closeness in action between the cytochrome P450 system and cytochrome c oxidase (COX) may indicate a similar xenobiotic biodegradation role to cytochrome c oxidase (COX), but at the meat level, which is, of course, exhausted after certain postmortem periods, as with other antioxidants. Also, cytochrome *c* oxidase (COX) activates the caspase system, which initiates apoptosis [35]. The presence of postmortem cascades, particularly endogenous

proteolytic system that has similar action to that of biotransformation occurring premortem, it could be assumed that such postmortem proteolytic system could contribute to hydrolytic cleavage of various antibiotic-residue bonds, resulting in the formation of various reaction products. Verification of this concept should involve the detection of numerous reaction products. The recent obvious reduction in screened residues that happened 48 hours postmortem supports the theory, but because of the limits of the current analysis, this hypothesis still has to be thoroughly investigated in the future. Moreover, it should be noted that not all drugs examined for this type of action exhibited a significant drop in the 48-hour-rigor-mortem residue level. This could be due to the structural characteristics of each antibiotic. Finally, if such cascades were tested in vivo and proved to influence residues, further research could give a potential preventive and control strategy for antibiotic residues and their associated public health risks.

Conclusions

The substantial drop in the descriptive pH48h values of the ribeye samples as compared to the mean pH4h indicates that the rigor mortis process was started in the meat samples. The current research findings reveal that a 48-hour rigor-mortis with chilling at 4 °C has a statistically significant decreasing impact on five antibiotic residues in meat tissues, except of amoxicillin, which is reduced in the sum of meat and kidney samples. The current study suggests that postmortem meat fluid loss, such as purge loss, and the presence of similar antibiotic residue biotransformation under rigor-mortem interacting cascades, contribute to the residues declining curve between 4 h and 48 h postmortem. However, considering the limitations of the present study, future research involving many antibiotic-positive samples should center around these recommendations, with a particular emphasis on identifying reaction products that could be produced postmortem from proposed postmortem biotransformation cascades, such as hydrolytic cleavage of various antibiotic residues. Finally, conduct experiments on animals using known specific antibiotics to validate and/or rule out this possibility.

List of abbreviations

Antimicrobial use (AMU), antimicrobial resistance (AMR), liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography (GC), capillary electrophoresis (CE), enzyme-linked immunosorbent assay (ELISA), liquid chromatography (LC), postmortem (PM), adenosine triphosphate (ATP), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glycogen phosphorylase (PYGM), heat shock proteins (HSPs), superoxide dismutase (SOD), beta-enolase (ENO3), phosphoglucomutase-1 (PGM1), protein-L-isoaspartate O-methyltransferase (PCMT1), proteasome subunit beta type-2 (PSMB2), *Musculus longissimus thoracis et lumborum* (*LTL*), Liquid chromatography-tandem mass spectrometry analytical method (LC-MS/MS), solid-phase extraction (SPE), Electrospray Ionization (ESI), Multiple Reaction Monitoring (MRM), reactive oxygen species (ROS), electron transport chain (ETC), tricarboxylic acid cycle (TCA), cytochrome c oxidase (COX).

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Conflicts of interest

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TABLE 1. Demonstrate 4- and 48-hour postmortem pH of ribeye samples dedicated to assessing Rigor Mortis duration effect on antibiotic residues.

	Minimum	Maximum	Mean	SEM	*P value*
pH_{4h}	5.755	6.17	6.025	0.0325	0.002
pH_{48h}	5.695	6.04	5.87	0.0292	

Min, Minimum, Max, Maximum, SEM, mean standard error.

TABLE 2. Illustrate effect of 48 h of Rigor-mortis on chilled ribeye meat antibiotic residue levels

Antibiotic (ng/gm)	4hr postmortem				48hr postmortem				*P* value
	Min	Max	Mean ± SEM		Min	Max	Mean ± SEM		
Amoxicillin	106.57	459.61	296.30[a]	33.38	58.33	264.39	153.46[b]	15.06	0.005
Ampicillin	5.78	23.19	12.67[a]	1.65	3.06	13.68	6.83[b]	1.00	0.044
Cefotaxime	109.07	127.35	118.21	3.34	87.26	101.88	94.57	2.67	0.181
Ciprofloxacin	97.42	237.38	165.07[a]	15.01	53.76	122.15	78.56[b]	7.90	0.039
Danofloxacin	6.91	49.15	28.27	4.18	4.64	32.84	16.94	2.66	0.117
Enrofloxacin	74.89	270.58	173.35	20.59	44.12	115.88	87.13	7.11	0.052
Norfloxacin	4.00	33.12	20.13[a]	2.64	1.62	17.68	10.97[b]	1.37	0.022
Oxytetracycline	68.00	401.00	216.38	26.01	28.44	227.91	122.75	16.10	0.058
Tylosin	166.08	484.99	374.6[a]	31.38	75.51	321.52	229.73[b]	23.60	0.017
Sulfonamides	4.08	33.79	19.0	2.60	3.00	27.03	14.74	2.08	0.400
Gentamycin	75.34	469.29	277.05	44.47	37.03	309.68	146.81	24.72	0.083
Florfenicol	9.96	27.31	19.78	2.29	4.82	16.98	10.42	1.34	0.118

Min, Minimum, Max, Maximum, SEM, mean standard error.

TABLE 3. Illustrate effect of 48 h of Rigor mortis on chilled kidney antibiotic residue levels

Antibiotic (ng/gm)	4hr postmortem				48hr postmortem				*P* value
	Min	Max	Mean ± SEM		Min	Max	Mean ± SEM		
Amoxicillin	29.32	380.32	165.52	28.06	22.99	322.11	133.80	23.87	0.491
Ampicillin	37.94	322.39	185.56	26.49	28.55	237.22	149.44	21.20	0.450
Cefotaxime	31.53	240.61	152.86	14.80	22.81	211.83	126.62	15.95	0.382
Ciprofloxacin	34.73	306.11	193.75	24.05	26.23	251.89	151.07	20.56	0.375
Gentamicin	16.62	345.13	158.41	29.13	12.50	301.44	130.46	24.72	0.626
Sulfonamides	27.44	300.86	173.54	24.72	11.18	232.80	99.21	19.74	0.148

Min, Minimum, Max, Maximum, SEM, mean standard error.

TABLE 4. The levels of different antibiotic residue positively detected in both chilled ribeye meat and kidney samples (*n* = 30) at 4- and 48-h **postmortem periods.**

Antibiotic (ng/gm)	4hr postmortem				48hr postmortem				*P* value
	Min	Max	Mean ± SEM		Min	Max	Mean ± SEM		
Amoxicillin	29.32	459.61	230.91[a]	34.65	22.99	322.11	143.63[b]	19.60	0.016
Ampicillin	5.78	322.39	99.11	29.33	3.06	237.22	78.13	23.91	0.571
Cefotaxime	31.53	240.61	147.08	13.86	22.81	211.83	119.50	14.32	0.265
Ciprofloxacin	34.73	306.11	183.32	20.70	26.23	251.89	124.70	19.02	0.089
Gentamicin	16.62	469.29	221.69	40.04	12.50	309.68	139.18	23.92	0.088
Sulfonamides	4.08	300.86	90.32	26.21	3.00	232.80	56.97	17.61	0.323

Min, Minimum, Max, Maximum, SEM, mean standard error.

References

1. Canton, L., Lanusse, C. and Moreno, L. Rational Pharmacotherapy in Infectious Diseases: Issues Related to Drug Residues in Edible Animal Tissues. *Animals*, **11**, 1–32 (2021).
2. Wang, B., Xie, K. and Lee, K. Veterinary Drug Residues in Animal-Derived Foods: Sample Preparation and Analytical Methods. *Foods,* **10**(3),555 (2021).
3. Khalifa, H. O., Shikoray, L., Mohamed, M.-Y. I., Habib, I. and Matsumoto, T. Veterinary Drug Residues in the Food Chain as an Emerging Public Health Threat: Sources, Analytical Methods, Health Impacts, and Preventive Measures. *Foods,* **13**, 1629 (2024).
4. Caneschi, A., Bardhi, A., Barbarossa, A. and Zaghini, A. The Use of Antibiotics and Antimicrobial Resistance in Veterinary Medicine, a Complex Phenomenon: A Narrative Review. *Antibiotics,* **12**, 487 (2023).
5. Getahun, M., Abebe, R. B., Sendekie, A. K., Woldeyohanis, A. E. and Kasahun, A. E. Evaluation of antibiotics residues in milk and meat using different analytical methods. *International Journal of Analytical Chemistry,* **2023**, 380261 (2023).
6. Okocha, R. C., Olatoye, I. O. and Adedeji, O. B. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Reviews,* **39**, 21 (2018).
7. Courtenay, M., Castro-Sanchez, E., Fitzpatrick, M., Gallagher, R., Lim, R. and Morris, G. Tackling antimicrobial resistance 2019–2024 – The UK's five-year national action plan. *Journal of Hospital Infection,* **101**, 426–427 (2019).
8. Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., Han, C., Bisignano, C., Rao, P. and Wool, E. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet,* **399**, 629–655 (2022).
9. World Health Organization. A one health priority research agenda for antimicrobial resistance. (2023).
10. Flynn, K., Villarreal, B. P., Barranco, A., Belc, N., Björnsdóttir, B., Fusco, V., Rainieri, S., Smaradóttir, S. E., Smeu, I., Teixeira, P. and Jörundsdóttir, H. Ó. An introduction to current food safety needs. *Trends in Food Science & Technology,* **84,** 1–3 (2019).
11. Góchez, D., Raicek, M., Pinto Ferreira, J., Jeannin, M., Moulin, G. and Erlacher-Vindel, E. OIE Annual Report on Antimicrobial Agents Intended for Use in Animals: Methods Used. *Frontiers in Veterinary Science,* **6**, 317 (2019).
12. Ding, Z., Wei, Q., Liu, C., Zhang, H. and Huang, F. The Quality Changes and Proteomic Analysis of Cattle Muscle Postmortem during Rigor Mortis. *Foods,* **11**, 217 (2022).
13. Matarneh, S., England, E., Scheffler, T. and Gerrard, D. in *Fidel Lawrie's Meat Science,* 159–185 (Woodhead Publishing, 2017).
14. Ding, Z., Wei, Q., Zhang, C., Zhang, H. and Huang, F. Influence of oxidation on heat shock protein 27 translocation, caspase-3 and calpain activities and myofibrils degradation in postmortem beef muscles. *Food Chemistry,* **340,** 127914 (2021).
15. Malheiros, J. M., Braga, C. P., Grove, R. A., Ribeiro, F. A., Calkins, C. R., Adamec, J. and Chardulo, L. A. L. Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Science,* **148**, 64–71 (2019).
16. Yu, Q., Wu, W., Tian, X., Hou, M., Dai, R. and Li, X. Unraveling proteome changes of Holstein beef M. semitendinosus and its relationship to meat discoloration during post-mortem storage analyzed by label-free mass spectrometry. *Journal of Proteomics,* **154**, 85–93 (2017).
17. O'brien, J., Campbell, N. and Conaghan, T. Effect of cooking and cold storage on biologically active antibiotic residues in meat. *Epidemiology & Infection,* **87**, 511–523 (1981).
18. Barros, S. C., Silva, A. S. and Torres, D. Multiresidues Multiclass Analytical Methods for Determination of Antibiotics in Animal Origin Food: A Critical Analysis. *Antibiotics,* **12**, 202 (2023).
19. Elsheikh, M., Osman, A., Edris, S., Dawam, W., Sitohy, M. and Sabeq, I. Soybean Glycinin's Antibacterial Properties Provide a Feasible Natural Alternative for Improving the Overall Quality and Shelf-Life of Beef Steaks and Combating Foodborne Pathogens. *Food and Bioprocess Technology,* (2024). doi:10.1007/s11947-024-03557-z
20. Arfa, I., Edris, S., Ahmed‑Farid, O. and Sabeq, I. Critically important antibiotic-residues assessment in raw meat of various origins marketed in Egyptian markets by mass spectrometer. *Benha Veterinary Medical Journal,* **47**, In press, (2025).
21. Shackelford, S. D., Koohmaraie, M. and Savell, J. W. Evaluation of Longissimus dorsi muscle pH at three hours Post mortem as a predictor of beef tenderness. *Meat Science,* **37**, 195–204 (1994).
22. White, A., O'Sullivan, A., Troy, D. J. and O'Neill, E. E. Manipulation of the pre-rigor glycolytic behaviour of bovine M. longissimus dorsi in order to identify causes of inconsistencies in tenderness. *Meat Science,* **73**, 151–156 (2006).
23. Yan, T., Hou, C., Wang, Z., Li, X., Chen, L., Liang, C., Xu, Y. and Zhang, D. Effects of chilling rate on progression of rigor mortis in postmortem lamb meat. *Food Chemistry,* **373**, 131463 (2022).
24. Adegbeye, M. J., Adetuyi, B. O., Igirigi, A. I., Adisa, A., Palangi, V., Aiyedun, S., Alvarado-Ramírez, E. R., Elghandour, M. M. M. Y., Molina, O. M., Oladipo, A. A. and Salem, A. Z. M. Comprehensive insights into antibiotic residues in livestock products: Distribution, factors, challenges, opportunities, and implications for food safety and public health. *Food Control,* **163**, 110545 (2024).
25. Gamil, B., Salem, A. M., Arab, W. S. and Sabeq, I. I. The microbiological quality, shelf-life, and multidrug-resistant Salmonella contamination rates assessment in chicken giblets purchased from live poultry shops. *The Microbe,* **3**, 100057 (1–7) (2024).

26. Huff-Lonergan, E. and Lonergan, S. M. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science,* **71**, 194–204 (2005).

27. Cars, O. Pharmacokinetics of antibiotics in tissues and tissue fluids: a review. *Scandinavian Journal of Infectious Diseases,* **22**(*suppl.* 74), 23–33 (1991).

28. Rizk, M., Zou, L., Savic, R. and Dooley, K. Importance of Drug Pharmacokinetics at the Site of Action. *Clinical and Translational Science,* **10**, 133–142 (2017).

29. Puccini, V. Antibiotic Therapy and Athletes: Is the Mitochondrial Dysfunction the Real Achilles' Heel? *Sports,* **10**, 131 (2022).

30. Bhat, Z. F., Morton, J. D., Mason, S. L. and Bekhit, A. E.-D. A. Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness,* **7**, 196–204 (2018).

31. Xue, M., Huang, F., Huang, M. and Zhou, G. Influence of oxidation on myofibrillar proteins degradation from bovine via μ-calpain. *Food Chemistry,* **134**, 106–112 (2012).

32. Kuriyama, T., Karasawa, T. and Williams, D. W. in *Biofilms in Infection Prevention and Control* (eds. Percival, S. L., Williams, D. W., Randle, J. & Cooper, T.) 209–244 (Academic Press, 2014). doi:10.1016/B978-0-12-397043-5.00013-X

33. Phang-Lyn, S. and Llerena, V. A. Biochemistry, biotransformation. StatPearls Publishing [internet], *Treasure Island (FL)* (2023).

34. Pedersen, K. W., Hansen, J., Hasselstrøm, J. B. and Jornil, J. R. Stability investigations of cytochrome P450 (CYP) enzymes immediately after death in a pig model support the applicability of postmortem hepatic CYP quantification. *Pharmacology Research & Perspectives,* **9**, e00860 (2021).

35. Morse, P. T., Pérez-Mejías, G., Wan, J., Turner, A. A., Márquez, I., Kalpage, H. A., Vaishnav, A., Zurek, M. P., Huettemann, P. P., Kim, K., Arroum, T., De la Rosa, M. A., Chowdhury, D. D., Lee, I., Brunzelle, J. S., Sanderson, T. H., Malek, M. H., Meierhofer, D., Edwards, B. F. P., Díaz-Moreno, I. and Hüttemann, M. Cytochrome c lysine acetylation regulates cellular respiration and cell death in ischemic skeletal muscle. *Nature Communications,* **14**, 4166 (2023).

تأثير التسلسلات البيولوجية بعد الذبح على مستوى متبقيات المضادات الحيوية ذات الأهمية القصوى في اللحوم المصرية

اسلام عرفة[١]، شيماء نبوي ادريس [١]،عمر آحمد فريد [٢] و اسلام إبراهيم سابق[١]

[١] قسم الرقابة الصحية على الاغذية – كلية الطب البيطري – جامعة بنها - القليوبية - مصر.

[٢] الهيئة القومية للرقابه علي الادوية - مصر.

الملخص

تهدف الدراسة الحالية إلى تقييم آثار الفترات المختلفة من التسلسلات البيولوجية للتحلل الرمي بعد الذبح ، التي تتراوح من أربع إلى ثمان وأربعين ساعة، على بقايا المضادات الحيوية الموجودة في عينات لحم الريب والكلى من الماشية المسوقة في الأسواق المصرية. فرضية الدراسة هو القدرة القوية لبعض احداث التحلل الرمي على تعديل وحتى تحليل مكونات اللحوم الهيكلية، مما يؤثر ويحسن جودة اللحوم. يشير الانخفاض الكبير في جميع القيم الوصفية لـلاس الهيدروجيني المسجل بعد ٤٨ ساعة لعينات لحم الريب مقارنة بمتوسط قيم لـلاس الهيدروجيني المسجل بعد أربع ساعات، إلى أن عينات لحم الريب خضعت بشكل واضح لأحداث التحلل الرمي. وفقا للبحث الحالي، تم تحديد ١٢ من متبقيات المضادات الحيوية فقط من أصل اثنين وثلاثين من المضادات الحيوية تم تحليلها باستخدام تحليل الكروماتوغرافيا السائلة مع مطيافية الكتلة (LC-MS/MS) في العضلات والكلى. يُظهر التحلل الرمي لمدة 48 ساعة مع التبريد عند ٤ درجات مئوية تأثيرات تقليلييه ذات دلالة إحصائية على متبقيات المضادات الحيوية في أنسجة اللحم البقري، بما في ذلك الأموكسيسيلين والأمبيسيلين والسيبروفلوكساسين والنورفلوكساسين والأوكسي تتراسيكلين والتايلوسين ($P < 0.05$). ولوحظ وجود اتجاه تنازلي صغير بين مستويات متبقيات المضادات الحيوية المقيمة بعد الذبح بأربع ساعات والأخرى المقيمة بعد ثمان واربعون ساعة مثل متبقيات الانروفلوكساسين والأوكسي تتراسيكلين والجنتاميسين ($P > 0.05$). في المقابل، لم تتأثر متبقيات المضادات الحيوية في الكلى بفترة التحلل الرمي البالغة ثمان واربعون ساعة. وفقًا للدراسة الحالية، من المحتمل أن يكون انخفاض منحنى متبقيات المضادات الحيوية بين أربع وثمانية وأربعين ساعة بعد الذبح ناجمًا عن فقدان سوائل اللحوم بعد الذبح، و/او احتمال حدوث تحولات او اسْتِقْلابات حيوية لمتبقيات المضادات الحيوية في ظل مسارات التحلل الرمي مماثله لما يحدث في حياه الحيوان من عملية الاسْتِقْلاب للتخلص من متبقيات المضادات الحيوية. ومع ذلك، ونظرًا لقيود الدراسة الحالية، يجب أن تكون هذه الاقتراحات هي محور الأبحاث المستقبلية التي تتضمن عددًا كبيرًا من العينات الإيجابية للمضادات الحيوية، والتركيز على اكتشاف منتجات التفاعل التي قد تنجم عن الاسْتِقْلاب الحيوي بعد الوفاة مثل الانقسام المائي لبقايا المضادات الحيوية المختلفة. و/أو أخيرًا إجراء تجارب على الحيوانات المزودة بمضادات حيوية محددة معروفة لتأكيد و/أو استبعاد مثل هذا الاحتمال.

الكلمات الدالة: التحلل الرمي، متبقيات المضادات الحيوية، لحم الريب والكلى، الآس الهيدروجيني، محلات الجزاره.