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Research Article

Presence of Antibiotic Residues of Penicillin and Oxytetracycline in Beef, Mutton and Chicken (Meat): One Health Requires Paradigm Shift Legislation and Policy Formulation

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Antibiotic Resistance, One Health, Public Health, Drug Law, Food Policy, Food Safety

Abstract | Nevertheless, with the discovery and increased production of pharmaceuticals, there has been a substantial use of pharmaceutical products that include antibiotics, steroid hormones. The day by day increase in human population across the globe is leading to the increased need to produce food. The high value of livestock products and their increasing demand creates a niche for the consumption of a variety of livestock products, which contribute to the food security (Lindahl *et al*. 2019). Due to the excessive use of common antibiotics (penicillin and oxytetracycline), the antibiotic residues remain in livestock products like meat (beef, mutton and chicken), it leads to antibiotic resistance which is a problem for one health. Following were the objectives of the current study: Detection of commonly used antibiotics (penicillin and oxytetracycline) in mutton, chicken and beef as a one health issue. High Performance Liquid Chromatography (HPLC) method was used on the samples collected from Lahore district of Punjab, Pakistan. The chicken samples were 28.33%, beef samples were 23.33% and mutton samples being sold in open market were 20% positive respectively. Thus, this makes it an indispensable question to be explored and figured out further prospectively to mitigate this very emerging issue of one-health tremendous need at national and international level.

Novelty Statement | Detection of antibiotic (AB) residues of commonly used antibiotics (ABs) penicillin and oxytetracycline in different categories (species) of meat through HPLC in Lahore has been first time studied.

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Introduction

As a matter of fact, with the discovery of pharmaceuticals, there has been tremendous use of pharmaceutical products that includes antibiotics (ABs) and steroidal hormones. Moreover, the day by day increase in human population across the globe is leading towards increased need of food production (Raza *et al.*, 2022). Due to the excessive use of common antibiotics (penicillin and oxytetracycline), the antibiotic residues remain in livestock products like meat i.e., beef, mutton and chicken in a categorical manner (Fatoki *et al.*, 2018).

The concept of 'One Health' (OH) ties together the wellbeing of people, animals, and the environment (Acharya *et al.*, 2020). One Health, a term used recently, refers to the improvement of the health including all biotic and abiotic factors (Kardjadj and Ben-Mahdi, 2019). The One World-One Health figures out emerging human and animal health issues (Miró and López-Vélez, 2018). The ABs have been being used; this has lead towards one health issues (Lu *et al.*, 2019). The study has revealed that the issues which are related to the accumulation of antibiotics residues from different sources is becoming part of wastewater are eventually leading to the antibiotic resistance (AR) (Sorinolu *et al.*, 2021). The clinical waste product systems increase the risk of antibiotic resistance (Voigt *et al.*, 2019). Similarly, The ABs are tremendously used in humans along with veterinary sectors (Lu *et al.*, 2019).

The veterinary medicines and drugs abuse has grave implications on the public health and as a matter of fact, eventually on one health due to the non-implementation of withdrawal periods (Pugajeva *et al.*, 2019). ABs are often used to prevent and treat illnesses. These ABs are also used to enhance the growth rate of livestock animals and birds. Such a tremendous use of veterinary drugs in animals results into the deposition of antimicrobial (AMRs) residues. Moreover, a major fraction remains as residue in milk, meat, and other dairy products Antibiotic usage in animals has impacts for human health (Bacanlı and Başaran, 2019). The human beings can be protected by controlling the undesirable ABs residues in meat through the significant efforts (Kyriakides *et al.*, 2020). It is dire need of era to understand the Antibiotic residues of common ABs like penicillin and oxytetracycline in beef, mutton and chicken so that local representatives, academics, policymakers, and medical practitioners may conduct dialogue and collaborate for the achievement of OH (Yasmeen *et al.*, 2021).

We conducted a study to identify antibiotics (penicillin and oxytetracycline) in meat that was marketed for the human consumption in Lahore based on previous research reporting the widespread use and abuse of antibiotics in animals. This is the first report from Pakistan having key objectives to detect commonly used antibiotics like penicillin and oxytetracycline in beef, mutton and chicken as a one health issue and suggesting a way-forward for the legislatives to ensure food safety through food controlling authorities.

Materials and Methods

Selection of sampling area

The samples of beef, mutton and chicken were collected from various open market of Lahore, district of Punjab province, Pakistan. Baad-Shahi Mosque to Sundar Estate Area, these areas included from North to South and Johar Town to DHA, these areas included from East to West of Lahore district of Punjab, Pakistan.

Samples parameters, collection, preservation and processing

The experimental source included raw meat in forms of (i) beef, (ii) mutton and (iii) chicken. Sixty (60) samples comprising fifty (50) grams per sample making a total of 180 samples were collected from the open markets of Lahore district of Punjab province, Pakistan.

The sample was collected in plastic zipper bag that was transported in ice boxes to the laboratory of Department of Clinical Medicine, University of Veterinary and Animal Sciences Lahore. For preservation purpose, the samples were kept in refrigerator prior to the testing. The samples were processed according on protocol described by (Teixeira *et al.*, 2020). An HPLC procedure was used to quantify the ABs residues (Figure 1).

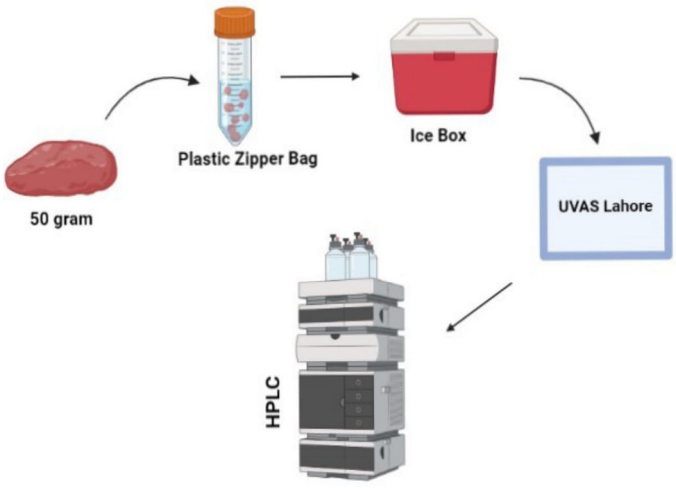

Figure 1: HPLC performance to quality the Abs residues.

Statistical analyses

SPSS (IBM Corp., NY, USA) version 26.0 was used to encode the data set. Reports of baseline characteristic frequencies were made. All variables were originally investigated using a univariable analysis to identify the risk factors linked to the incidence of antibiotic residues in samples. The correlation between antibiotic residue and the positive samples was evaluated using Chi-square (χ^2). Multivariable logistic regression (MLR) was also employed to identify factor linked with positive and negative dichotomous sample outcome. The 95% Confidence Interval (C.I.) was used to emphasize the odds ratio in the MLR-based statistical test, which was based on the two-sided Wald test.

Results and Discussion

Overall results

Respectively, there were 14, 12 and 17 samples of beef, mutton and chicken positive out of 60 total samples from each category. Beef being sold in open market 23.33%, mutton being sold in open market 20.00% and chicken being sold in open market 28.33% were positive respectively lowering down from highest 28.33 % to lowest 20%. This difference of percentage is above 20% and below 30% for all categories which means that their number of positive samples have no significant difference of percentage. These results have indicated that antibiotics are mostly used in chicken. Furthermore, Chi-square based statistical analysis is presented as a whole result (Tables 1, 2 and Figures 2, 3).

Table 1: Meat categories revealing positive and negative samples along with percentages.

S. No.	Category	Positive	Negative	Positive %	Negative %
1.	Beef sold in open market	14	46	23.33	76.67
2.	Mutton sold in open market	12	48	20	80
3.	Chicken sold in open market	17	43	28.33	71.67
4.	Total	43	137	23.89	76.11

Table 2: Overall statistical summary of results of meat samples.

Chi-square tests	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	4.700[a]	4	0.319
Likelihood ratio	4.810	4	0.307
Linear-by-linear association	1.555	1	0.212
N of valid cases	1000		

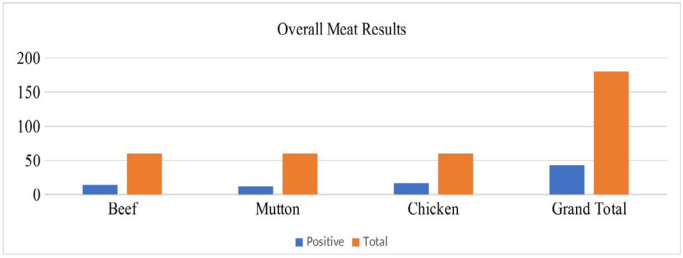

Figure 2: Overall summary of results of meat samples positive against Abs in numbers.

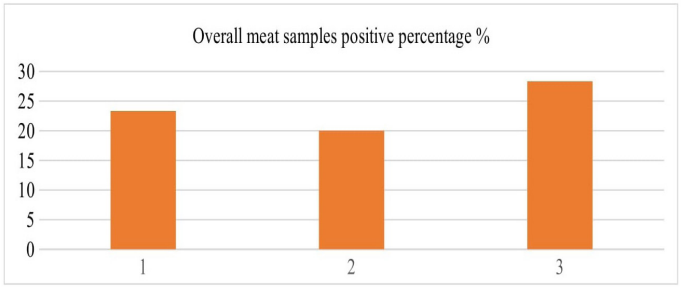

Figure 3: Percentage overall meat samples positive against Abs residues.

Presence of penicillin and oxytetracycline (µg/kg) in meat samples- quantification of positive samples

In beef type of meat the highest penicillin residues were detected as 13.95µg/Kg lowest penicillin residues were detected as 7.98µg/Kg, average penicillin residues were detected as 10.24 µg/Kg , the highest oxytetracycline residues were detected as 296.07µg/Kg, the lowest oxytetracycline residues were detected as 188.62µg/Kg, average oxytetracycline residues were detected as 240.16 µg/Kg, the highest both penicillin and oxytetracycline simultaneously residues were detected as 12.72 and 296.07µg/Kg, the lowest both penicillin and oxytetracycline residues were detected as 10.07 and 188.62µg/Kg and average both penicillin and oxytetracycline residues were detected as 11.40 and 262.53µg/Kg. In mutton samples, the highest penicillin 13.27 µg/Kg, lowest penicillin 8.29 µg/Kg, average penicillin 10.34 µg/Kg, the highest oxytetracycline 290.12 µg/Kg, the lowest oxytetracycline 198.84 µg/Kg, average oxytetracycline 260.90µg/Kg, the highest both penicillin and oxytetracycline simultaneously 12.72 and 272.12 µg/Kg, the lowest both penicillin and oxytetracycline were 8.86 and 244.49 µg/Kg and average both penicillin and oxytetracycline 10.57 and 256.17 µg/Kg or ng/ were, respectively detected as antibiotics residues.

In chicken type of meat the highest penicillin 13.12 µg/Kg or ng/g, lowest penicillin 7.66µg/Kg, average penicillin 10.53 µg/Kg, the highest oxytetracycline 310.73 µg/Kg, the lowest oxytetracycline 194.72 µg/Kg, average oxytetracycline 247.17 µg/Kg, the highest both penicillin and oxytetracycline simultaneously 10.19 and 255.37 µg/Kg, the lowest both penicillin and oxytetracycline 9.87 and 217.93 µg/Kg, average both penicillin and oxytetracycline

were 10.03 and 236.65 µg/Kg were respectively detected as antibiotics residues (Table 3).

Table 3: Delineating penicillin and oxytetracycline in meat samples µg/kg or ng/g.

Drugs in meat	Beef meat	Mutton meat	Chicken meat
Highest Penicillin	13.95	13.27	13.12
Lowest Penicillin	7.98	8.29	7.66
Average Penicillin	10.24	10.34	10.53
Highest Oxytetracycline	296.07	290.12	310.73
Lowest Oxytetracycline	188.62	198.84	194.72
Average Oxytetracycline	240.16	260.90	247.17
Highest both Penicillin and Oxytetracycline	12.72 and 296.07	12.72 and 272.12	10.19 and 255.37
Lowest both Penicillin and Oxytetracycline	10.07 and 188.62	8.86 and 244.49	9.87 and 217.93
Average both Penicillin and Oxytetracycline	11.40 and 262.53	10.57 and 256.17	10.03 and 236.65

Association of meat with common antibiotics

When HPLC performed then there were 7/14, 5/12 and 8/17 against penicillin while 5/14, 4/12 and 7/17 against oxytetracycline and 2/14, 3/12 and 2/17 against both (penicillin and oxytetracycline) of beef, mutton and chicken (Table 4).

Table 4: Association of categorically of meat samples with common antibiotics.

Antibiotics	Group	Positive	Negative	χ2	Df	p-value
Penicillin	Beef	7 (16.27)	7 (16.27)			
	Mutton	5 (11.62)	7 (16.27)	119.4	2	<0.001
	Chicken	8 (18.60)	9 (20.93)			
Oxytetra-cycline	Beef	5 (11.63)	9 (20.93)	105.6	2	<0.001
	Mutton	4 (9.30)	8 (18.60)			
	Chicken	7 (16.28)	10 (23.26)			
Both	Beef	2 (04.65)	12 (27.91)	112.2	2	<0.001
	Mutton	3 (06.98)	9 (20.93)			
	Chicken	2 (04.65)	15 (34.88)			

Meat samples positive against penicillin

The samples of meat were found positive against Penicillin i.e., (i) beef 7 (11.67%), (ii) mutton 5 (8.33%) and (iii) chicken 8 (13.33%) being sold in open. Chicken was found the highest positive against penicillin and mutton as the lowest. The statistical analyses have revealed It means that the positive samples had similar quantity of penicillin among aforementioned various categories of meat i.e., beef, mutton and chicken being sold in open market (Tables 5, 6, 7 and Figure 4).

Table 5: Meat samples positive against penicillin along with percentages.

S. No.	Meat samples	Penicillin	Percentage %
1.	Beef	7	11.67
2.	Mutton	5	8.33
3.	Chicken	8	13.33

Table 6: Meat samples positive against penicillin through ANOVA.

Mean	10.493	10.427	10.431
Standard deviation	1.882	1.772	1.636
F value	0.0000001		
p value	0.996		

Table 7: Meat samples positive against penicillin through chi-square Chi-square tests.

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	0.788[a]	2	0.675
Likelihood ratio	0.811	2	0.667
Linear-by-linear association	0.084	1	0.772
N of valid cases	180		

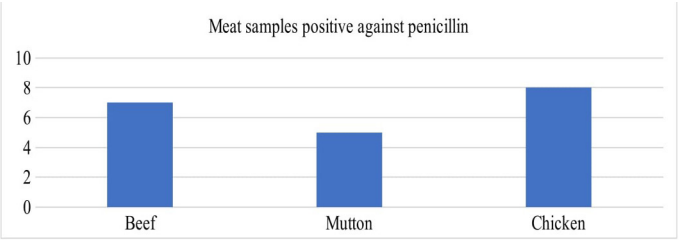

Figure 4: Number of meat samples positive against penicillin.

Meat samples positive against oxytetracycline

The samples of meat were found positive against Penicillin i.e., (i) beef 5 (8.33%), (ii) mutton 4 (6.67%) and (iii) chicken 7 (11.67%) being sold in open. Chicken was found the highest positive against penicillin and mutton as the lowest. ANOVA based statics have depicted that insignificant difference have been figured out among all of the aforementioned categories in quantification of positive meat samples. The p-value obtained through Chi-square was 0.619 for the aforementioned 3 categories of meat only for the positive samples which have been quantified against oxytetracycline and it depicts that insignificant difference (Tables 8, 9, 10 and Figure 5).

Table 8: Meat samples positive against oxytetracycline along with percentages.

S. No.	Meat samples	Oxytetracycline	Percentage %
1.	Beef	5	8.33
2.	Mutton	4	6.67
3.	Chicken	7	11.67

Table 9: ANOVA results of oxytetracycline positive samples in categories of meat.

MEAN	246.5	258.9	244.8
Standard deviation	46.2	30.8	42.2
F value	0.27		
p value	0.769		

Table 10: Chi-Square results of meat samples positive against oxytetracycline.

Chi-Square Tests	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	0.960[a]	2	.619
Likelihood ratio	0.946	2	.623
Linear-by-linear association	0.409	1	.522
N of valid cases	180		

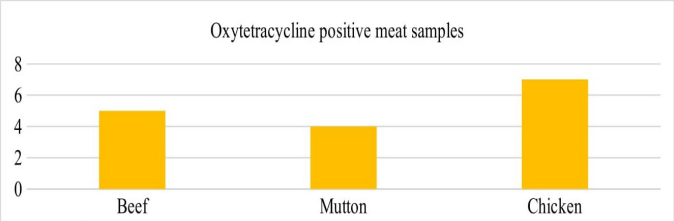

Figure 5: Meat samples positive against oxytetracycline in numbers.

Meat samples positive against both penicillin and oxytetracycline simultaneously

The samples of meat were found positive against Penicillin i.e., (i) beef 2 (3.333%), (ii) mutton 3 (5%) and (iii) chicken 2 (3.333%) being sold in open. Chicken was found the highest positive against penicillin and mutton as the lowest. The p-value obtained via Ch-square test was 0.862 for the aforementioned 3 categories of meat only for the positive samples which have been quantified against Oxytetracycline and it has depicted insignificant difference (Tables 11, 12 and Figure 6).

Table 11: Meat samples positive against both penicillin and oxytetracycline simultaneously.

S. No.	Meat samples	Penicillin	Percentage %
1.	Beef	2	3.333
2.	Mutton	3	5
3.	Chicken	2	3.333

Table 12: Meat samples positive against both penicillin and oxytetracycline simultaneously Chi-Square tests.

	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	0.297[a]	2	.862
Likelihood ratio	0.286	2	.867
Linear-by-linear association	0.000	1	1.000
N of valid cases	180		

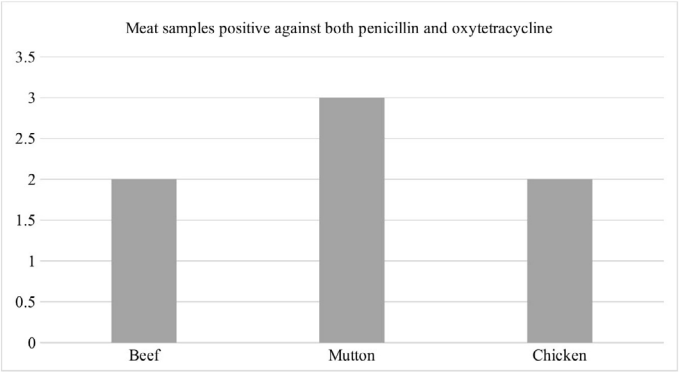

Figure 6: Meat samples positive against both penicillin and oxytetracycline simultaneously in numbers.

The samples are presented as ± standard deviation error. The p-value equated 0.996 in the 3 categories mentioned above of meat samples. This indicates that insignificant differences are considered between measuring positive meat samples. It means that samples had the same amount of penicillin between different categories. The p-value obtained remained 0.319 for the 3 types of meat and it had shown the slightest difference between all of the clauses mentioned above. In simple terms, it means that samples have the same amount of penicillin and oxytetracycline.

Food safety- a global public health requirement

The contaminating of food by chemicals is a concern for global public health and a primary source of international trade issues. Meat containing antibiotic residues that enters the food chain without an appropriate withdrawal period exposes consumers to a variety of health risks, including allergic responses, various forms of toxicity, and antibiotic resistance. Lack of information may enhance the likelihood of taking veterinary drug residues via animal products. Meat that has been cooked reduces the danger linked with antibiotic consumption. For a more precise risk estimate, more research utilizing cocked meat and a large sample of households is required. Despite this, the findings of this study demonstrate conclusively that meat consumption poses a significant danger.

Presence of Abs residues in livestock products- a grave threat to one health

Our findings about the presence of Abs residue in livestock products are close to the findings of (Lee *et al*., 2018) and (Pérez-Valdespino *et al*., 2021) who reported Abs residues in meat, milk, and eggs. Similarly, After the analysis, veterinary antimicrobials (AMs) were detected in meat (Kyriakides *et al*., 2020) reported. Current study reported penicillin and oxytetracycline have been quantified but these findings might be different in different scenario due to difference of number of samples as well as the categories of samples i.e., eggs have not been the part of current study. Antibiotic residue contents in some samples were more than 50ng/g and as high as 148.4ng/g and 3629.2ng/ml for chloramphenicol

in meat and milk. These findings are similar in context of presence of antibiotics, however, during the current studies penicillin and oxytetracycline have been figured while Wang *et al*. (2017) have indicated the presence of chloramphenicol. Oxytetracycline (OTC) is the most often used antibiotic within the tetracycline category in veterinary medicine. Uncontrolled use of these antibiotics for treatment and prevention will lead to a buildup of these antibiotics or their metabolites in numerous tissues, such as muscles, liver, and kidneys (Wang *et al*., 2017).

Identification and quantification of Abs in food to ensure OH

In consideration of public health and food safety, it is vital to identify and quantify antibiotic residues in various animal-derived food items and to educate the public about their negative consequences. The government and associated authorities must enforce veterinary medication usage restrictions. In addition, the deployment of procedures to restrict the prescription and dispensing of antimicrobial (AM) agents to licensed veterinarians as well as the execution of educational programs aimed at users and consumers are required. Additional research on AM residues is required to bolster veterinary medication surveillance strategies. This study strongly encourages collaboration between food safety specialists, animal and human healthcare professionals, and politicians to ensure implement appropriate surveillance of antimicrobial usage and to preserve powerful AM fit for future generations' disease prevention. In order to ensure the health of the residents of Lahore region in particular and Pakistan, the result of current study might be utilized by the government as a guide for instituting new regulations to limit the existing danger. It is necessary to impose stringent regulations on the use of antimicrobial drugs in the livestock industry and to analyze animal-derived food sources prior to commercialization.

Conclusions and Recommendations

Presence of lethal levels of Abs in human's consumed meat

The data regarding the presence of commonly used ABs i.e., penicillin and oxytetracycline in livestock which are used as routine food for human consumption has depicted lethal levels of antibiotic residues in raw meat categories of (i) beef, (ii) mutton and (iii) chicken.

Outcomes, measures taken and recommendations

As per following the current studies aims have been obtained which could be extended prospectively by discovering new avenues through multidisciplinary vistas:

1. Grave kind of effects of antibiotic residues were figured out from the analyzed data which were present in categories of meat like beef, mutton and chicken on national food safety and public health eventually lead to one-health issue. This is because of Abs abuse in livestock which remains unmeasured at the hands of quackery.
2. The information above has helped to recommend realistic next steps for various stakeholders. These stakeholders include legislative, judiciary, corporate sector, common public (consumers) and prescription policy makers.
3. Public awareness-based newspapers article has been published in national dailies and awareness campaigns have been made through national television channels.

The veterinary antimicrobials (AMs) should be strictly controlled the authorities concerned to protect public health (Kyriakides *et al*., 2020). The abuse or misuse of veterinary drugs has grave kind of implications on public health and as a matter of fact, eventually on one health. Such kind of food is unsuitable for human consumption. The tenacious and strict ABs residues control in food is essential to assure the human health (Pugajeva *et al*., 2019).

Pragmatic way forward

The sagacious and prudent efforts should be made both at public health level and animal health level eventually to maintain the one health. The multi-disciplinary collaboration would be indispensable to undertake the monitoring operations (Humboldt-Dachroeden *et al*., 2020). Since many of the research scholars have delineated the issue and the same have been endorsed through current studies. The livestock products especially beef, mutton and chicken are consumed by the public at large. The livestock-based food containing residues of active pharmaceuticals can damage public health, animal health and environment. As a result, this serious problem threatens the three pillars of health-animals, people, and the environment. This issue needs to be further explored from a variety of perspectives like presence of different Abs, types of animal foodstuff samples and also antibiotic resistance caused by the presence of Abs residues in foodstuff at national and international level.

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Ethics approval and consent to participate

Not applicable in current studies, however, the research proposal has been approved by the Syndicate and Advance Studies Research Board (ASRB), UVAS, Lahore.

Availability of data and materials

Consequent upon request, the corresponding author will provide the numerical data.

Conflict of interest

The authors have declared no conflict of interest.

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