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Impact of cooking methods of meat on antibiotic residues: Review

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Abstract
Antibiotics are the most conventionally used veterinary drug for raising food animals. They are used so, to ward off the undesirable effects generated by the multiple infectious pathogens. It helps to maintain the optimum level of production in farm animals without falling ill. The consequences faced with the use of drugs lead to the generation of antibiotic residues in the food products, consumed by the public. These residues will put forth a complication to the health of humans when it exceeds above the limit of the Maximum residues limit (MRL). The presence of antibiotic residues in animal-derived foods is one of the major causes of the development of antibiotic resistance in human pathogens. To overcome the antibiotic residues in food products nowadays lots of researches going on besides that, several methods of cooking like frying, boiling, roasting, grilling, etc., are also known to have reduced the level of antibiotic and other drug residues in the meat products. In this paper, we explore some antibiotic residues in meat and meat products and their reduction levels while applying several cooking methods.

Keywords: Antibiotic residues, cooking methods, withdrawal period, maximum residual level, tolerable limit

Introduction
Meat from the food animals is a valuable source of protein, minerals, and other nutrients and widely accepted as one of important food products, worldwide. Rising non-vegetarianism is generally due to increased income and the subsequent diversification of diets for better and greater protein intake (Muthukumar *et al.*, 2017) [31]. Owing to the expanding population of the human race, food production needs to be increased. To satisfy this emerging demand for the production of meat and its allied products, greater enhancements in production and productivity are expected day by day. The goal of achieving production of increased quantity of meat, egg, or milk at the lower cost led to the intensive animal production, where animals and birds are confined at high stocking density to ensure greater output with lowest input (Gliessman 2015) [11]. The modern integrated and intensified poultry/livestock production necessitates the use of several agrochemicals, especially antibiotics and other veterinary drugs to contain the diseases, enhance growth promotion for producing food commodities as cheaply and efficiently as possible to meet demands of an ever expanding world population (Maged and Hamdey, 2006 [27]; Muthukumar and Mandal, 2017) [30]. However, indiscriminate use of veterinary drugs and antimicrobials in livestock/poultry without following required withdrawal periods leads to accumulation of their residues in the tissues and organs of treated poultry/livestock and eventually become part of the human food chain. Residues of antibiotics remaining in animal-derived human foods may pose potential human health hazards toxicologically, microbiologically or immuno-pathologically. The matter therefore, assumes greater significance to monitor the presence of these residues in meat at regular intervals to ensure that public health is not compromised by violate residues (Noel *et al*., 2005) [35]. The presence of these residues above the permissible level is also a major bottleneck in the acceptance of food commodities by the importing countries. The importing countries impose legal restrictions with respect to residues in the food. Concerning international trade and consumer trust, residues of drugs, and/or their prospective metabolites in animal derived-foods are significant (Kalpana *et al*., 2012) [23].

Poultry industry

The meat production from poultry is 4.06 million tonnes which contribute about 50% of the total meat production in India (8.11 million tonnes) (BAHS, 2019) [3]. Antibiotics are used, notably in broilers, for disease prevention which in turn indirectly helps to accelerate the growth. It can increase the rate of growth through various means, such as thinning of mucous membranes in the gut to promote absorption, enhancing intestinal motility, facilitating an ideal environment for beneficial gut flora through the destruction of pathogenic organisms, suppressing cytokine for better muscle growth (Nisha, 2008) [34].

The worldwide annual average intake of antimicrobials per kilogram of raised animals is 172 mg/kg, 148 mg/kg, and 45 mg/kg for pigs, chicken, and beef respectively. The global antimicrobial consumption will increase by 67% between 2010 and 2030, from 63,151 ± 1,560 tonnes to 105,596 ± 3,605 tonnes (Van Boeckel *et al.*, 2015) [46].

Impacts of residues on public health

The antibiotic residues from the food animals affect health in several ways. The direct sequel on the health of consumers includes bone marrow toxicity (chloramphenicol), nephropathy (gentamicin), reproductive disorders, allergy, or hypersensitivity reactions (beta-lactam antibiotics), etc. (Goulette, 2007) [14]. Another sequel will be the indirect effect owing to the emergence of antibiotic resistance (Glynn *et al.*, 1998 [12]; Butaye *et al.*, 2001) [5]. It is worrying that these estimates will be more than 10 million by 2050 than the current figure of 700,000 individuals losing their life to antimicrobial resistance fight each year by 2050. Besides, antimicrobial resistance is becoming the reason that people suffer more than the overall victims of cancer and road accidents (O'Neill, 2014) [36]. An increased risk of colon cancer (first and middle portions) is associated with the penicillin class of drugs, whereas tetracycline is associated with cancer in the last segment of the colon (Lo *et al*., 2002) [25]. Continuous ingestion of meat or meat products contaminated with toxic drug residues induces biotransformation changes of endogenous and exogenous compounds, resulting in several health issues, namely neurological disorders, carcinomas, and endocrine dysfunction (Muthukumar and Mandal, 2017) [30].

Table 1: Level of antibiotic residues in animal products

Antibiotic	Specimen	Residue level (ppb)	Reference
Ciprofloxacin	Chicken muscle	89.60	Ramatla *et al*., 2017 [39]
	Chicken liver	152.20	
	Cattle muscle	89.60	
	Cattle liver	145.20	
	Cattle Kidney	98.20	
	Pig muscle	42.60	
	Pig liver	220.00	
	Pig Kidney	72.50	
Chloramphenicol	Chicken	12.64-226.62	Yibar *et al.*, 2011 [49]
Enrofloxacin	Chicken liver	10-10690	Sultan, 2014 [45]
Tetracycline	Cattle liver	30	Ramatla *et al*., 2017 [39]
	Sheep liver	20	
	Cattle muscle	46.8-220.2	
	Cattle – tissue	176.3	Abbasi *et al*., 2012 [1]
	Diaphragm	96.8	
	Kidney	672.40	
Oxytetracycline	Cured meat	42-360	Senyuva *et al.*, 2000 [43]
Sulphonamides	Chicken muscle	35.2-81.6	Ramatla *et al.*, 2017 [39]
	Chicken liver	20.7-65.9	
	Pig-liver	48.20-69.90	
	Pig-kidney	52.80-92.80	
	Chicken- muscle	0.02-0.8	Mehtabuddin *et al*., 2012 [28]
Doxycycline	Chicken- muscle	847.7	Jank *et al*., 2017 [21]
Streptomycin	Chicken -muscle	98.44-452.90	Ramatla *et al*., 2017 [39]
	Cattle –muscle	625.90-989.20	
	Pig-muscle	620.30-875.80	

R- Residue level in Raw meat (µg/kg), C- Residue level in Cooked (µg/kg), R%- Reduction % in residue level, C%-change in residue %, B-boiling, Br- barbecuing, R- roasting, G- grilling, M- microwaving, F –Freezing, N- sample size

Enrofloxacin

Enrofloxacin belongs to a potent class of antibiotics (fluoroquinolone) that are prevalently used in veterinary and human medicine. They are effective against gram-positive, gram-negative, and Mycoplasma organisms. Fluoroquinolone exhibits concentration-dependent action, i.e., depending on the concentration of the drug, the duration, and intensity of bacterial lysis takes place. (Kalpana *et al.*, 2015) [24].

Quinolones are very stable during thermal procedures, with almost no results obtained even by heating to ultra-high temperatures (shaltout *et al.*, 2019) [44]. Enrofloxacin residues are not affected by cooking procedures however, an apparent decrease in enrofloxacin concentration is observed as some of the liquid used for cooking (boiling and frying) was lost by exudation. In contrast, an apparent increase in residue concentration is observed in a water-loss cooking technique (roasting and grilling) (Lolo *et al.*, (2006) [26]. Frying is an effective way of reducing enrofloxacin residue concentrations in chicken meat (Hassan *et al.*, 2019) [16].

Table 2: Impact of cooking methods on enrofloxacin residues

Sample	N	Enrofloxacin			References
		R	C	% C	
Chicken leg	4	50.2	26.07(B)	-48.07	Lolo *et al*., 2006 [26]
			25.62(F)	-48.97	
			22.5(M)	-55.18	
			102.91(R)	105	
			75.00 (G)	49.40	
Chicken Breast		17.8	7.94 (B)	-55.40	
			12.22(F)	-31.35	
			9.43(M)	-47.03	
			32.06 (R)	80.11	
			28.33(G)	59.15	
Chicken Thigh	3	27.2	19.5 (B)	-28.3	Hassan *et al*., 2019 [16]
			11.6 (F)	-57.4	
			8.3 (G)	-69.5	

R- Residue level in Raw meat (µg/kg), C- Residue level in Cooked (µg/kg), R%- Reduction % in residue level, C%-change in residue %, B-boiling, Br- barbecuing, R- roasting, G- grilling, M-microwaving, F –Freezing, N- sample size.

Ciprofloxacin

Ciprofloxacin also belongs to fluoroquinolones, which are reported to be a group of synthetic antimicrobial agents that are highly effective against diverse microbial infections having a broad range of actions. They are known to act by inhibiting DNA gyrase, which during cell division affecting the stability of the bacterial DNA molecule's DNA configuration. These are widely used in humans to treat urinary tract and enteric infections. It is primarily used in farm animals for the treatment and prevention of infectious diseases.

The heat treatment methods of boiling, grilling, and roasting do not affect the degradation of ciprofloxacin residues. As ciprofloxacin is a heat-stable compound that gets degrade less by the heat treatment process, microwaving and freezing will greatly reduce the ciprofloxacin residue (Hasanen *et al*., 2016 [15]; Gogoi and Roy, 2019) [13] still freezing is the efficient method to degrade ciprofloxacin residues to a safe level than microwaving (Fahim, 2019 [8]; Shaltout *et al.*, 2019) [44].

Table 3: Impact of cooking methods on ciprofloxacin residues

Sample	N	Ciprofloxacin			References
		R	C	R%	
Chicken Thigh	3	193.7	4.3 (M)	97.8	Hasanen *et al*., 2016 [15]
			6.8 (F)	96.5	
			193.7(B)	0	
			193.7(G)	0	
Chicken Breast	3	204.2	6.5 (M)	96.8	
			8.2 (F)	96	
			204.2(B)	0	
			204.2(G)	0	
Rabbit Meat	3	56.4	44.7(B)	20.7	Fahim, 2019 [8]
			34.9(M)	38.1	
			49.18(R)	12.8	
			19.34(F)	65.7	
Chicken	6	895 1217907	597 (B)	43.3	Gogoi and Roy, 2019 [13]
			570 (F)	53.2	
			264(M)	70.9	
Chicken	45	388	301(B)	22.4	Shaltout *et al.*, 2019 [44]
			250(M)	35.5	
			322(R)	17.0	
			145(F)	62.6	

Sulfonamides

Sulfonamides are considered the synthetic antimicrobial agents acting as competitive dihydropteroate synthetase enzyme inhibitors. They are used to treat several gastrointestinal and urinary tract infections (Igwe and Okoro, 2014) [19].

Autoclaving of the incurred piglet muscle tissue results in a maximum reduction (29.6%) of sulfamethazine followed by the boiling (18%), while the microwave (15%) is the least significant (Papapanagiotou *et al*., 2005) [37]. Fish fillets when cooked at 190 °C contributed to an average 54.0 percent decrease in ormetoprim and a 46.1 percent decrease in sulfadimethoxine (Xu *et al.*, 1996) [48].

Sulfonamide reduction has a greater effect on the time duration factor (9, 6, and 3 min) than the deep frying temperature of chicken meatballs (170, 180, and 190 °C). For sulfaquinoxaline, sulfamethazine, sulfadiazine, sulfamethoxazole respectively, the maximum reductions obtained are 27.6, 27.5, 37.5, and 40.7 percent (Ismail-Fitry *et al.*, 2008) [20]. Similarly in chicken meatballs, residues of sulfadiazine, sulfaquinoxaline, sulfamonomethoxine, and sulfamethoxazole results in decreased residue levels in boiling (45-61%), roasting (38-40%), and microwaving (35-41%) (Furusawa and Hanabusa, 2002) [10].

Tylosin

Tylosin, a macrolide group of antibiotics (broad-spectrum), has a specific action on *Mycoplasma* species. It is a combination of four macrolide antibiotics (Tylosin-A, B, C, D) formed by *Streptomyces fradiae*. Cooking methods, cooking temperature and length, and concentration of tylosin before processing are the factors that influenced tylosin residue in chicken meatballs during cooking. The overall decrease in tylosin will be 35.3 percent during microwave treatment (2 minutes) and 79.9 percent during boiling (30 minutes). By limiting the use of meat juice, tylosin residues might be minimized (Salaramoli *et al*., 2016) [42].

Tetracyclines

Tetracyclines are considered as the major antibiotics used, including oxytetracycline (OTC), chlortetracycline (CTC), tetracycline (TC), doxycycline (DOC). They are affordable and reported to have a broad spectrum of activity to Gram-positive and negative organisms. Usually, veterinarians recommend them for medicinal, prophylaxis, and growth promotion purposes in chicken and livestock by injections, feedstuffs, or drinking water. They are recommended for use in a wide variety of livestock, including chicken (Nguyen *et al*., 2013) [33].

Boiling is more effective in decreasing the concentration of OTC in muscle, while roasting was more effective in decreasing the concentration of broiler bird liver samples (Vivienne *et al.*, 2018) [47]. In water, OTC is found to be less temperature-stable than in cooking oil (Rose *et al.*, 1996) [40]. Baking at 145 °C (20 minutes) will be enough to kill almost 55 percent of the initial amount of TC residues in chicken, (Said and Salma, 2019) [41]. The cooking process, time, and temperature can play a significant role in reducing TC antibiotic residue while cooking food among the different agents affecting antibiotic residue after the cooking process (Javadi, 2011) [22].

Table 4: Impact of cooking methods on tetracyclines residues

Sample	N	Oxytetracycline			References
		R	C	R%	
Lamb	2	6470	330 (B)	95	Ibrahim, 1994 [18]
		5690	4700 (F)	17.3	
Chicken	3	1914	294 (B)	84.6	Abou-Raya *et al*., 2013 [2]
		1790	18 (M)	99	
		1650	89 (R)	94.6	
Chicken	120	446.16	69.04 (B)	84.52	Elbagory *et al*., 2016 [7]
			28.46 (F)	93.62	
			15.25 (G)	96.58	
Chicken	3	3.81	2.47 (B)	35.17	Hussein *et al*., 2016 [17]
			1.96 (F)	48.55	
			0.98 (M)	74.27	
Chicken Thigh	40	11190	4585 (B)	59	Nashwa *et al.*, 2016 [32]
			6592 (R)	41.1	
			2142 (M)	80.9	
Chicken Breast		4194	2392 (B)	42.3	
			3297 (R)	31.4	
			1032 (M)	75.4	
Beef	60	322.2	71.10 (B)	77.9	Mgonja *et al.*, 2018 [29]
			93.7 (Br)	70.9	
Rabbit Meat	3	27.2	3.27 (B)	87.97	Fahim, 2019 [8]
			3.54 (M)	86.95	
			7.07 (R)	73.98	
Chicken	45	244	53.84 (B)	77.93	Shaltout *et al.*, 2019 [44]
			45.2 (M)	81.48	
			91.29 (R)	37.41	
			239 (F)	2.05	

Miscellaneous residues – Anthelmintic drugs

In the prophylaxis and treatment of endoparasitic and ectoparasitic infestations, a diverse range of anthelmintic drugs is being used in livestock. Different benzimidazole compounds (fenbendazole, triclabendazole, mebendazole, and albendazole), imidazothiazoles (levamisole), macrocyclic lactones (ivermectin) are used to manage nematode, cestode, and trematode infestations. Anthelmintic drug residues are generally resistant to degradation during shallow frying and roasting. Traditional cooking cannot be regarded as a precaution against the ingestion of veterinary anthelmintic drug residues in beef (Cooper *et al.*, 2011) [7].

Table 5: Impact of cooking methods on tilmicosin residues

Sample	N	Tilmicosin			References
		R	C	R%	
Chicken Thigh	3	1.45	0.92 (B)	36.5	Hussein *et al*., 2016 [17]
			0.78 (F)	46.4	
			0.86 (M)	0.86	

Table 6: Impact of cooking methods on ampicillin residues

Sample	N	Ampicillin			References
		R	C	R%	
Chicken	120	253.7	47.6 (B)	81.2	Elbagory *et al*., 2016 [7]
			24.10 (F)	90.5	
			13.9 (M)	94.5	

Table 7: Impact of cooking methods on gentamicin residues

Sample	N	Gentamicin			References
		R	C	R%	
Chicken Thigh	3	2.56	1.64 (B)	35.9	Hussein *et al*., 2016 [17]
			1.29 (F)	49.6	
			1.12 (M)	56.2	

Reason for the reduction of antibiotic residues level in cooked meat

Transfer of antibiotic residues from the muscle to the boiling water and loss of juices that come from the muscle as it is roasted may explain the reduction of antibiotic residue after different cooking techniques. (Furusawa and Hanabusa, 2002 [10]; Lolo *et al*., 2006 [26]; Nguyen *et al*., 2013 [33]; Shaltout *et al*., 2019 [44]; Javadi, 2011) [22]. Cooking methods cannot guarantee the complete removal of the drugs found in meat and can only reduce the concentrations of these drug residues to a safer level. By discarding any juices that come from the edible tissues as they are cooked, exposure to antibiotic residues can be minimized (Javadi, 2011) [22].

Strategies to turn down the antibiotic residues

The first and foremost step is to create awareness among individuals and organizations by the way of education through veterinary personnel, literature, and government authorities. System for national control and monitoring of the use, resistance, and residues of veterinary antibiotics need to be carried out. The analysis, grading, and forbidding of product possessing residues, exceeding the Maximum residues limit (MRL) is done by adopting rapid screening procedures (Nisha, 2008 [34]; Muthukumar and Mandal, 2017 [30]; Biswas *et al*., 2019) [4].

Antibiotics use should be avoided before the slaughter of animals, if necessary arise withdrawal period recommended for the specific antibiotics should be strictly followed. Avoid the use of broad-spectrum and long-acting antibiotics as a growth promoter in food animals. Further sub-therapeutic and overdose of antibiotics to be avoided. Use of drugs with a minimal withdrawal period in food animals is recommended.

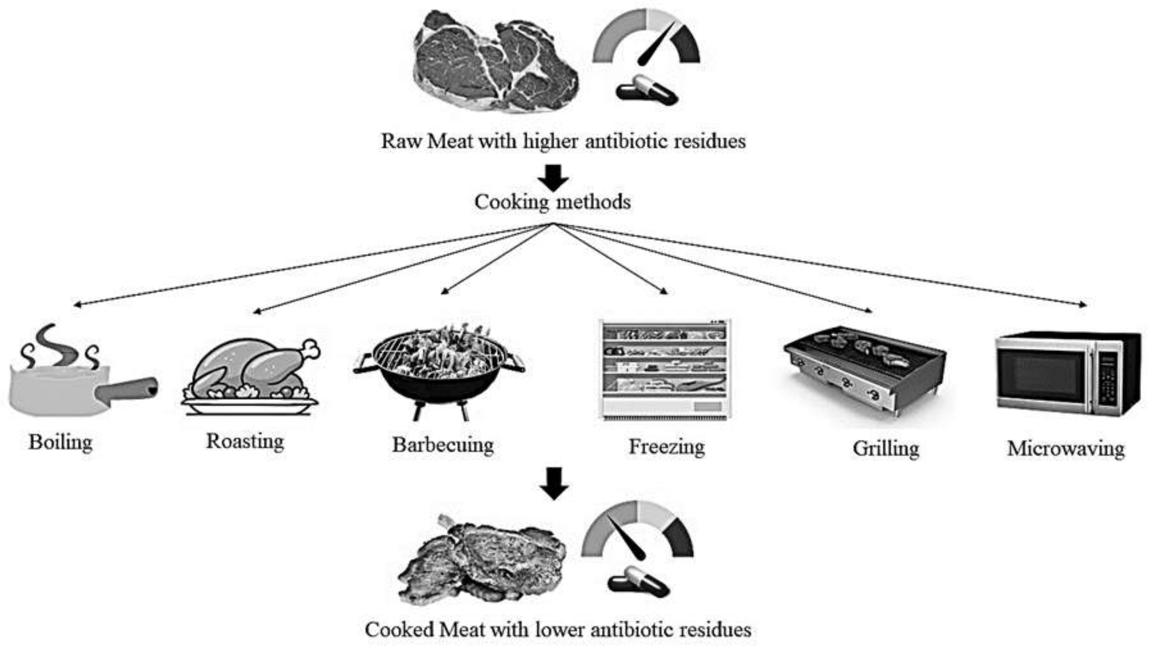

Fig 1: Flow chart indicating the effects of different cooking methods on the level of antibiotic residues

Table 8: Tolerable limits of antibiotic residues in meat (FSSAI 2011) [9]

S. No.	Antibiotics	Tolerance limit (mg/kg) in meat
1	Ampicillin	0.01
2	Cloxacillin	0.01
3	Chlortetracycline/Oxytetracycline/Tetracycline	0.2
4	Erythromycin	0.1
5	Trimethoprim	0.01
6	Enrofloxacin	0.01
7	Tilmicosin	0.15
8	Monensin	0.01
9	Tylosin	0.1
10	Meloxicam	0.01
11	Sulphadiazine	0.01
12	Flunixin Meglumine	0.01

(Prescott and Baggot, 1993) [38]

Table 9: Withdrawal period for various antibiotics in food animals

Antimicrobials	Withdrawal time before slaughter (Days)			
	Chicken/ poultry	Swine	Sheep	Cattle
Ampicillin	-	15	-	06
Amoxicillin	-	-	-	25
Oxytetracycline	05	28	-	03
Chlortetracycline	01	0	0	10
Tylosin	03	14		21
Procaine penicillin G	05	28	9	10
Sulfamethazine	-	15	-	07

Conclusion

In food-producing animals, the use of antibiotics has the potential to generate residues in animal-derived products and creates a health threat to the consumer. The most likely cause for drug residues could be human handling, such as inappropriate use of antibiotics and illegal drug applications, and not comply with the withdrawal period. The residues present in the food materials are mainly influenced by the parameters like the nature of the drug (heat-stable or liable), its residue level in raw meat, cooking method, time, and temperature. It is recommended to properly screen the raw meat for antibiotic residues before food processing. There will be a need for new diagnostic tests to monitor antibiotic residues and to develop new antimicrobials or antibiotics. The use of eubiotics *viz.*, probiotics, prebiotics, organic acids, and essential oils can be an alternative option to antibiotics and can be promoted. The development of organic production of food animals can be a protective means of eliminating antibiotic residues in meat.

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