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Article

Antibiotic resistance and residue in chicken, cattle, buffalo and goat meats in different southern districts of Bangladesh

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Abstract: The presence of antibiotic residue in chicken and animal meats is a serious threat to human health due to its harmful effects. This study aimed at identifying the antibiotic resistance patterns of the isolates as well as antibiotic residues in chicken, cattle, buffalo and goat meats in different southern districts of Bangladesh. A total of 205 meat samples, including 70 chicken meat, 60 cattle meat, 50 buffalo meat and 25 goat meat were aseptically collected and analysed for the detection of antibiotic residues by thin layer chromatography and the isolates obtained from these samples were subjected to antibiogram study against 16 commonly used antibiotics. The isolates found in this study were *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, and *Salmonella* spp. and their prevalence were 37.5% (77/205), 22.1% (48/205), 29.7% (61/205), 8.7% (19/205), respectively. The isolates showed different degrees of sensitivity to the antibiotics used in the study. The most resistant phenotype was against cefradine, amoxicillin, penicillin, oxytetracycline, erythromycin, and enrofloxacin. 18.5% (38/205) meat samples were found to be positive for antibiotic residues and the highest prevalence was observed in chicken meat compared to other meat types. Overall, the findings of the study suggest that it is important to take controlling measures for the emergence of antibiotic resistance and also for ensuring healthy meats for human consumption.

Keywords: antibiotic; resistance; residue; meat; TLC

1. Introduction

In veterinary medicine antibiotics are commonly used, therefore, foods derived from animals may contain antibiotic residues, which can adversely affect the human health (Chanda *et al.*, 2014). Also, administration of antibiotics to farm animals may impose certain hazards to human and animal health, including increased resistance of bacteria to antibiotics as well as allergic reaction (Walton 1988; Mathew *et al.*, 2001). However, worldwide farmers use antibiotics for therapeutic and prophylactic purposes, and also as growth promoter (Jinap *et al.*, 2010; Wadoum *et al.*, 2016). Therefore, food-producing animals remain as an important source of antimicrobial-resistant zoonotic bacteria (Michael *et al.*, 2017). In addition, indiscriminate use of antibiotics results residues in meat, milk, cheese, butter and other livestock products (Lee *et al.*, 2001). Antibiotics belonging to tetracycline, aminoglycoside, sulphonamide and potentiated sulphonamide, macrolide and lincosamide groups are commonly used as growth promoter at sub-therapeutic doses in poultry (Casewell *et al.*,

2003). Sulphonamides can also be used as additives in animal food, as prolonged ingestion of sulphonamides influence the growth-promoting effect (Long *et al.,* 1990). Tetracycline, a broad-spectrum antibiotic is used to treat infections as well as growth promoter in animals (Doyle *et al.,* 2006).

It is assumed that antibiotic residues in animal derived foods might be a potential source of human health hazards, and antibiotics resistance may significantly affect the health condition of both humans and animals. However, the antibiotic resistance of the isolates from different meat samples and presence of antibiotic residues in different meats are not well documented in Bangladesh. Therefore, in this study, we characterized the antibiotic resistance of the isolates obtained from different meat samples, including chicken meat, cattle meat, buffalo meat, and goat meat collected from different southern districts of Bangladesh. Also, we investigated the prevalence of antibiotic residues in these meat samples. The findings of the study should provide a documentation of the antibiotic resistance and antibiotic residues in meats of Bangladesh.

2. Materials and Methods

2.1. Collection of samples

A total of 205 meat samples, including 70 poultry meat, 60 cattle meat, 50 buffalo meat, and 25 goat meat were aseptically collected from three different southern districts of Bangladesh, including Barishal, Pirojpur and Bhola.

2.2. Transportation of samples

Samples were collected in sterile containers and transported to the laboratories maintaining the standard procedures. Each sample was divided into two aliquots, one aliquot was shipped to the laboratory of the Department of Microbiology and Public Health, Patuakhali Science and Technology University for isolation and characterization of isolates and another aliquot was shipped to the laboratory of the Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Chittagong for antibiotic residue detection by thin layer chromatography (TLC).

2.3. Isolation and identification of bacterial agents

Isolation of bacterial agents from the meat samples were performed by culturing the samples in different plates containing culture media, including eosin methylene blue (EMB) agar, MacConkey agar, xylose lysine deoxycholate (XLD) agar, salmonella and shigella agar (SS agar), mannitol salt agar (MS agar), nutrient agar (NA), blood agar (BA), strep agar and mueller-hinton agar. Plates were incubated at 37^0C for 24-48 hours under aerobic condition. From the pure culture bacterial agents were identified by studying colony characteristics, Gram staining reaction, hemolysis pattern and biochemical test as described by Merchant and Packer (1967) and Cheesbrough (1984).

2.4. Antibiogram study

To know the antibiotic sensitivity pattern of the isolates against different commonly used antibiotics, antibiotic sensitivity test was performed by Disc Diffusion test as described previously (Bauer *et al.*,1966). This method is suitable for the determination of an *in vitro* efficacy of antibiotics by calculating the zone of inhibition diameter, which are caused by diffusion of the agent into the medium surrounding the disc. Sixteen commercially available antibacterial agents (Himedia Laboratories, India) were selected for the purpose. Antibiotics used in this study and their concentration per disc and diameter of zone of inhibition used for interpreting the sensitivity of the isolates are shown in Table 1.

2.5. Detection of antibiotic residues in meat

Thin layer chromatography (TLC) was performed to detect the presence of drug residues in meat samples, according to the method described previously (Poppelka *et al.,* 2005). Briefly, meat samples were blended for 3 minutes and taken into Petri dishes. Using spatula .04" of blended meat sample was taken into beaker. Then, 10 ml of phosphate buffered solution was added and mixed well. Next, 2 ml of trichloroacetic was added to the solution and centrifuged at 7000 rpm for 15 minutes. After centrifugation, the supernatant was filtered using filter paper. Filtrate was collected in a beaker. Diethyl ether (equal volume of filtrate) was added and incubated for 10 min. Then extracts were evaporated until complete dry. And the dried sample was then reconstituted in 2 ml of methanol and acetone (1:1) and kept for 20 min to reach the solution upto the mark. Finally, TLC plate was dried for 5 min and observed under UV light chamber to detect the antibiotic residues in meat samples.

2.6. Data analysis
Descriptive analysis was performed. Data were collected and calculated to determine antibiotic resistance and the occurrence of antibiotic residues in poultry, cattle, buffalo and goat meats.

3. Results
3.1. Identification of bacterial agents
Among 205 meat samples, 77 (37.5%) were found to be positive for *Staphylococcus*spp.,48 (22.12%) were positive for *Streptococcus* spp.,19 (8.76%) were positive for *Salmonella* spp. and 61(29.76%) were positive for *Escherichia coli* (*E. coli*) (Figure 1).

3.2. Antibiotic resistance pattern of the bacterial agents
The antibiogram study revealed that the isolated *Staphylococcus* spp. were highly resistant to cefradine followed by amoxicillin, penicillin, chloramphenicol, erythromycin. The isolates of *Streptococcus* spp. were highly resistant to amoxicillin followed by cefradine, oxytetracycline, enrofloxacin, penicillin, erythromycin, cotrimoxazole. Isolated *E. coli* showed varying degrees of sensitivity to antibiotics used in this study with highest sensitivity to cefradine followed by amoxicillin and penicillin. Isolated *Salmonella* spp. showed highly resistance to cefradine, followed by penicillin and oxytetracycline (Table 2).

3.3. Antibiotic residues in meats
The overall prevalence of antibiotic residues in meats was 18.5% (38/205). In Barishal, prevalence of antibiotic residues in chicken, cattle, buffalo and goat meat was 37.5(15/40), 20.0 (6/30), 0.0(0/5) and 10.0% (1/10), respectively (Figure 2A). In Pirojpur, the prevalence of antibiotic residues in chicken, cattle, buffalo and goat meat was 40.0 (6/15), 13.3 (2/15), 0.0 (0/10) and 0.0% (0/7), respectively (Figure 2B). In Bhola, the prevalence of antibiotic residues in chicken, cattle, buffalo and goat meat was 20.0(3/15), 6.7 (1/15), 8.6(3/35) and 12.5% (1/8), respectively (Figure 2C).

Table 1. Antibacterial agents used for the investigation of antibiotic sensitivity pattern.

Antibacterial agents	Concentration(µg/disc)	Interpretation of results (Zone diameter in mm)		
		Resistant	Intermediate	Sensitive
Amoxicillin	10µg	≤ 11	12-14	≥ 15
Cefradine	30µg	≤ 12	13-15	≥16
Chloramphenicol	10µg	≤ 12	13-17	≥ 18
Ciprofloxacin	5µg	≤15	16-20	≥21
Colistin sulphate	10µg	≤8	9-11	≥12
Gentamicin	10µg	≤12	13-14	≥15
Oxytetracycline	25µg	≤15	16-25	≥26
Penicillin	10µg	≤11	12-14	≥15
Tetracycline	30µg	≤14	15-18	≥19
Vancomycin	30µg	≤14	15-16	≥17
Enrofloxacin	15µg	≤10	11-12	≥13
Erythromycin	15µg	≤13	14-15	≥16
Norfloxacin	15µg	≤15	16-17	≥18
Cotrimoxazole	25µg	≤14	15-16	≥17
Azithromycin	30µg	≤17	18-19	≥20
Tobramycin	10µg	≤15	16-17	≥18

µg = microgram, mm = millimeter, R= resistant, I= intermediately sensitive, S= sensitive

Table 2. Antibiotic resistance patterns of the isolated bacteria.

Antibiotics		***Streptococcus* spp.**	***Staphylococcus* spp.**	***E. coli***	***Salmonella* spp.**
Vancomycin	S	1	2	1	1
	I	12	11	7	6
	R	11	4	2	5
Tobramycin	S	17	10	10	11
	I	2	1	4	3
	R	0	2	3	4
Erythromycin	S	0	0	0	0
	I	9	4	6	6
	R	15	13	14	16
Azithromycin	S	7	3	3	5
	I	13	8	19	15
	R	3	2	4	3
Ciprofloxacin	S	11	6	9	7
	I	13	7	12	14
	R	0	0	2	0
Oxytetracycline	S	0	0	0	0
	I	5	6	1	2
	R	8	4	8	9
Cotrimoxazole	S	5	1	1	2
	I	8	2	4	4
	R	9	4	6	5
Chloramphenicol	S	11	6	4	3
	I	6	4	3	3
	R	0	0	0	0
Amoxicillin	S	0	0	0	0
	I	2	1	1	0
	R	19	7	13	8
Colistin	S	3	3	9	3
	I	11	8	18	10
	R	0	0	2	1
Penicillin	S	1	0	0	0
	I	14	6	1	1
	R	13	12	9	11
Gentamicin	S	51	12	10	8
	I	1	0	0	1
	R	0	0	0	0
Cefradine	S	0	0	0	0
	I	2	0	0	0
	R	14	9	14	11
Enrofloxacin	S	0	0	0	0
	I	15	7	11	8
	R	4	2	2	1
Norfloxacin	S	5	3	3	2
	I	19	13	6	5
	R	2	2	1	2
Tetracycline	S	4	5	1	0
	I	9	4	1	1
	R	4	3	2	1

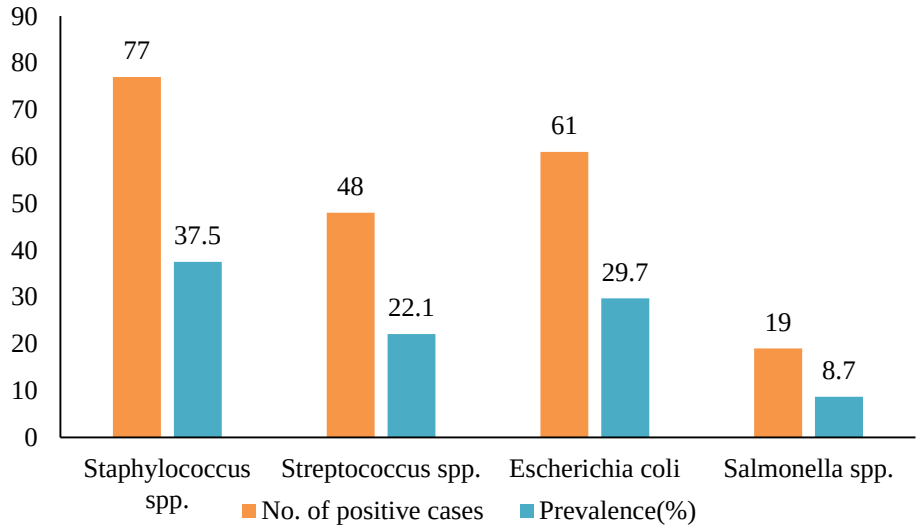
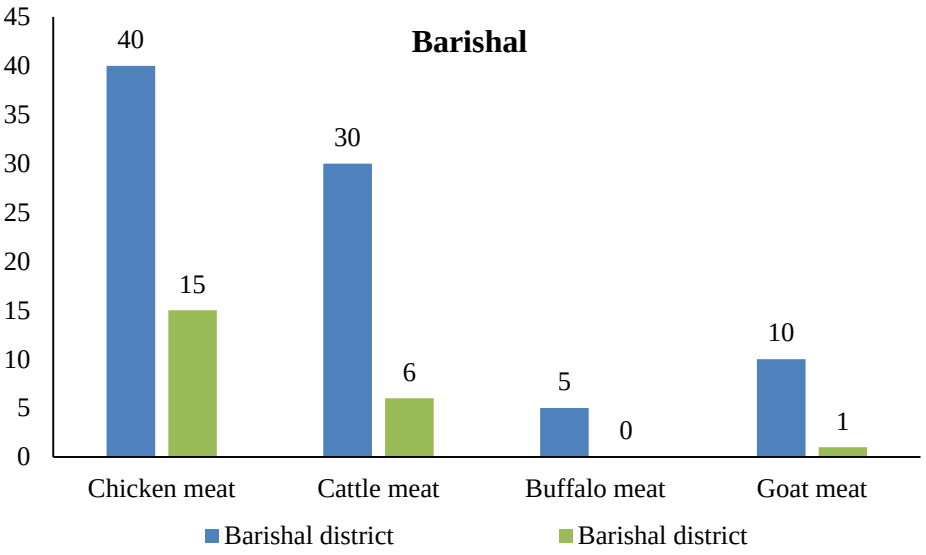


Figure 1. Prevalence of bacterial agents from meat samples.

A



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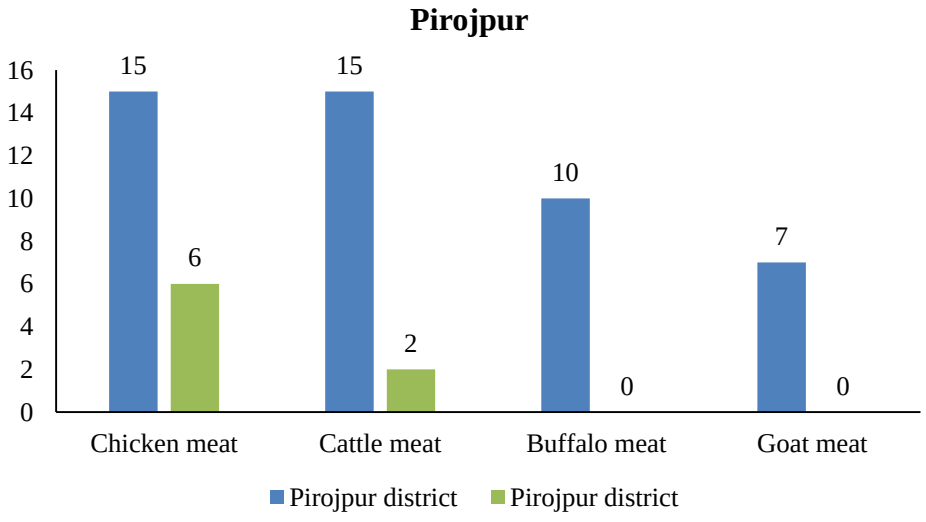

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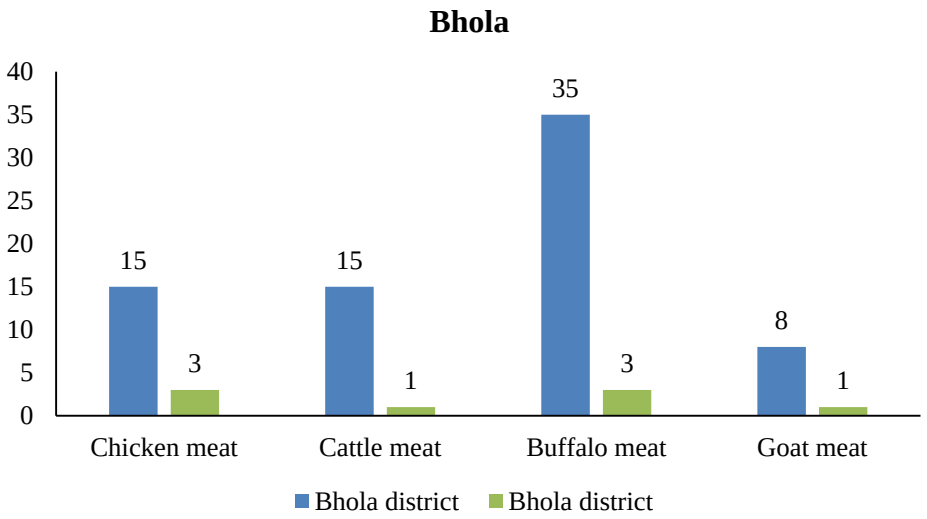

Figure 2. Detection of antibiotic residues in meats by using TLC in Barishal district (A), Pirojpur district (B), and Bhola district (C).

4. Discussion

In this study we determined the antibiotic resistance of the isolates obtained from chicken, cattle, buffalo and goat meat collected from different southern districts of Bangladesh. Also, we determined the prevalence of antibiotic residues in these meat samples. We observed that *Staphylococcus* isolates were highly resistant to amoxicillin, penicillin, chloramphenicol, erythromycin and cefradine. A recent study (Jahan *et al.*, 2015) found that *Staphylococcus* spp. were highly resistant to amoxicillin, penicillin, and erythromycin. The *Streptococcus* isolates were highly resistant to amoxicillin and oxytetracycline, followed by erythromycin, penicillin, and cotrimoxazole. A recent study (Cherazard *et al.*, 2017) showed that *Streptococcus* spp. were resistant to erythromycin and clindamycin. In our study we observed that *E. coli* showed varying degrees of sensitivity to different antibiotics and *E. coli* showed highly resistance to cefradine, followed by amoxicillin and penicillin, which is close to a recent study (Bhuvan *et al.*, 2019). In this study, isolated *Salmonella* spp. were highly resistant to amoxicillin and cefradine, followed by penicillin, erythromycin, and oxytetracycline. A study by Rahman *et al*. (2018) also observed that *Salmonella* spp. isolated from chicken meat were resistant to erythromycin, doxycycline, sulphonamide-trimethoprim, azithromycin, and oxytetracycline. In this study, we observed that the overall prevalence of antibiotic residues in meat was 18.5%. A previous study (Khan *et al.*, 2013) observed 21% overall prevalence of antibiotic residues in meat and another recent study (Rabin *et al.*, 2017) found 22.0%overall prevalence of antibiotic residues in meat. In Barishal, the prevalence of antibiotic residues in chicken meat was found 37.5%, which matched the findings of a previous study (Gebre *et al.*, 2012). In Pirojpur, the prevalence of antibiotic residues in chicken meat was found 40.0%, which was close to the findings of a previous report (Sarker *et al.*, 2018). In Bhola, the prevalence of antibiotic residues in chicken meat was 20.0%. Ramatla *et al*. (2017) observed 24.6% prevalence of antibiotic residues in chicken meat. In cattle meat, the prevalence of antibiotic residues in Barishal was 20.0%, which was almost similar to the findings of a previous study (Babapour *et al.*, 2012). The prevalence of antibiotic residues in Pirojpur was 13.3%, which supports the findings of a previous study (Sattar *et al.*, 2014). In our study the prevalence of antibiotic residues in Bhola was found 6.7%, whereas Nhung *et al.* (2018) reported 9.5% prevalence of antibiotic residues in cattle meat. In buffalo meat, the prevalence of antibiotic residues in Bhola was found 8.6%, but no antibiotic residue was found in buffalo meats of Barishal and Pirojpur district. A previous study (Khan *et al.*, 2013) reported 23.3% prevalence of antibiotic residues in buffalo meat. The prevalence of antibiotic residues in goat meat of Barishal and Bhola district was 10.0 and 12.5%, respectively. No antibiotic residue was found in goat meat of Pirojpur district. A previous study (Hossain *et al.*, 2011) reported 15.3%prevalence of antibiotic residues in goat meat.

5. Conclusions

In conclusion, the isolates obtained from the meat samples in this study showed varied degrees of sensitivity and resistance patterns toward different antibacterial agents. Antibiotic residues were relatively higher in poultry meat than that of other meat types in different southern districts of Bangladesh, which indicates more frequent

use of antibiotics in chicken meat production and is a potential threat to human health. The findings of the study will help to increase the awareness among the people regarding the use of antibiotics for healthy meat production.

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Conflict of interest
None to declare.

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