

## INCIDENCE OF ANTIBIOTIC RESISTANT BACTERIA IN POULTRY AND LIVESTOCK IN DHAKA CITY

Rahman M.H., Rahman M.M., Sultana R., and Kabir M.S.\*

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka

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Antimicrobial resistance is a concern for humans and animals all over the world. Indiscriminate use of antibiotics in livestock and poultry has become one of the major causes of antimicrobial resistance development in microorganisms. The aim of the study was to determine the antimicrobial resistance patterns of bacteria isolated from raw meat, intestine, feces and feed of farm chicken, cow and local chicken. Samples were collected from different retail shops at Malibagh area in Dhaka City, Bangladesh. Bacterial load was enumerated, potentially pathogenic bacteria were identified and antibiogram was determined following standard methods. A total of 43 bacterial isolates were identified from different samples which were *Escherichia coli*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Providencia* and *Acinetobacter* spp. Eight types of antibiotics such as, Ampicillin-10µg, Gentamicin-10µg, Amikacin-10µg, Amoxicillin-10µg, Ceftriaxone-30µg, Imipenem-10µg, Chloramphenicol-30µg and Tetracycline-30µg were used to determine the antibiotic sensitivity pattern of isolated bacteria. The antibiotic resistance pattern of bacteria isolated from feed and feces samples of cow and chicken were found to be similar. Isolated bacteria from chicken meats showed higher antimicrobial resistance (80-100%) against Ampicillin-10µg, Imipenem-10µg and Amoxicillin-10µg compared to cow meat isolates. The incidence of antibiotic resistant bacteria is a threat to animals, food handlers and consumers if they are being infected by these antibiotic resistant pathogenic bacteria.

**Keywords:** Antibiotic resistance, poultry, livestock, infection and human

## INTRODUCTION

Burden of antimicrobial resistant bacteria influences the economy and health of people in both developed and developing countries. Globally antimicrobials are becoming increasingly ineffective and posing as threats to both humans and animals (1-3). Antibiotic resistant bacteria like *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* spp., which are considered common human pathogens accounted for major food borne infections in many countries (4). Poultry and livestock are considered as reservoirs and beds for propagating such drug-resistant microorganisms (5). Foods prepared from animal sources can carry varieties of pathogenic and nonpathogenic microbes that have become a platform that helps in the evolution of new drug resistant and multidrug resistant (MDR) bacteria through the transfer and acquisition of drug-resistant genes (6, 7).

The extensive use of antimicrobials in the poultry industry for prevention of diseases and promotion of growth further triggers the mechanisms that lead to the emergence of drug-resistant bacteria (6, 7). Food borne diseases are now growing concern for public health all over the world which cause an estimated 48 million illnesses and 3,000 deaths in the United States and approximately 30 million illness in Bangladesh each year (8, 9). In developed countries, up to 30% of

the populace suffer from food borne illness every year, while in developing countries up to 2 million deaths are estimated to occur every year (10). This study was designed to investigate the presence of pathogenic microbes in feed, intestine, feces and meat of cow and chicken and determine their antimicrobial susceptibility profiles. In order to monitor and prevent the spread of antibiotic resistant pathogens, antimicrobial susceptibility pattern should be carefully monitored to promote appropriate use of antibiotics on poultry and farm animals.

## MATERIALS AND METHODS

**Study Design, Study Area and Sampling.** A cross-sectional study was carried out on farm chicken, cow and local chicken sold in Malibagh area of Dhaka city from October to December 2019. Feed, meat, intestine and feces samples were collected from farm chicken, cow and local chicken from the retail shops of this area. Feed, intestinal content, meat and feces were collected from respective samples and placed in sterile plastic bags and transported to the laboratory using cold transport box. Samples were collected following aseptic technique and brought to the Department of Microbiology, Stamford University Bangladesh for further analysis following standard methods (11).

**Total Plate Count (TPC).** For determining the bacterial load in samples, the total heterotrophic bacteria (THB), total coliforms (TC), total *Pseudomonas* spp. (TP) and total *Staphylococcus* spp. (TS) were determined following dilution and spread plate method. Nutrient agar, mFC agar, *Pseudomonas* agar and Mannitol Salt agar (Hi-Media Ltd., India) were used to determine the THB, TC, TP and TS in the collected samples, respectively. 10 g of each sample was added to 90 ml normal saline and homogenized for 2 minutes in a blender to make a solution. Each sample was serially diluted in 10-folds by adding 1.0 ml of homogenized sample to 9.0 ml sterile normal saline (0.85 % NaCl) and diluted from  $10^{-1}$  to  $10^{-6}$ . 100 µl from each dilution from each sample was spread on selected media by spread plate method. Plates were incubated for 24 hours at 37°C and characteristic colonies were counted and back calculated as CFU/g in log scale (12).

\*Corresponding Author: Mailing address. Dr. Md. Shahidul Kabir, Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh., E. mail: [kabir@stamforduniversity.edu.bd](mailto:kabir@stamforduniversity.edu.bd), Contact: +8801949622783.

**Isolation and Identification of Pathogenic Bacteria.** Different types of colonies from McConkey agar were further subcultured and biochemically identified by Kligler's Iron Agar, Motility Indole Urea, Citrate, Oxidase, Methyl Red and Voges Proskauer test (12).

**Isolation of *Shigella*, *Salmonella* and *Vibrio* spp.** Ten grams of each sample were homogenized for 2 minutes with 90 ml of distilled water. 1.0 ml of homogenized and diluted samples were inoculated to 9.0 ml of Selenite F broth and Alkaline peptone water and incubated at 37°C for 4-6 hours. After incubation broths were streaked onto SS agar and TCBS agar (Hi-Media Ltd., India) from the respective broths. The Presence of characteristics colonies of *Salmonella/Shigella* and *Vibrio* spp. were observed on SS agar and TCBS agar, respectively. The colonies were further identified by their morphological characteristics and biochemical properties following standard procedure (12).

**Antimicrobial Susceptibility Test.** Antibiotic sensitivity pattern of bacterial isolates was performed using Kirby Bauer Disk diffusion method on Mueller Hinton Agar (Hi-Media Ltd., India) according to the Clinical and Laboratory Standard Institution (CLSI) guidelines 2020. The antibiotic susceptibility pattern was examined by using commercial antibiotic discs including; Ampicillin-10µg (AMP-10), Gentamicin-10µg (GN-10), Amikacin-10µg (AK-10), Amoxicillin-10µg (AX-10), Ceftriaxone-30µg (CTX-30), Imipenem-10µg (IMP-10), Chloramphenicol-30µg (C-30) and Tetracycline-30µg (TE-30) (Hi-Media Ltd., India).

## RESULTS

**Total plate count (TPC).** The highest count of total bacteria was found in farm chicken samples. In case of feed sample higher bacterial load was found in farm chicken's feed ( $1.44 \times 10^8$  cfu/g). In case of intestine samples, higher bacterial load was found in farm

chicken intestine ( $1.0 \times 10^9$  cfu/g). Feces of local chicken and cow showed higher bacterial load ( $9.4 \times 10^9$  cfu/g). In case of meat samples higher bacterial load was found in farm chicken's meat ( $1.0 \times 10^9$  cfu/g) (Table 1).

**Isolation of bacteria from different sample.** The bacterial flora isolated from different cow, farm chicken and local chicken samples were presumptively identified as *Escherichia coli*, *Enterobacter*, *Citrobacter* spp., *Klebsiella*, *Providencia* and *Acinetobacter* spp. (Table 2).

**Antibiogram.** Based on the disc diffusion method bacteria were categorized as sensitive, resistance or intermediate (Table 3, 4 and 5) (12, 13). Out of eight antibiotics used this study most of the bacterial isolates were found to be sensitive to Gentamicin, Amikacin, Ceftriaxone, Chloramphenicol and Tetracycline. Almost all isolates (n = 43) demonstrated resistance against Amoxicillin. All isolates were found 100% resistance against Ampicillin and Imipenem (Figure 1). The value for each bar indicates the percentage of isolates showing resistance against respective antibiotic.

Table 1: Total bacterial load in different samples.

Sample	Type	cfu/gm				Present/Absent	
		THB	TC	TP	TS	<i>Salmonella/Shigella</i>	<i>Vibrio</i>
Farm chicken	Feed	$1.44 \times 10^8$	$3.6 \times 10^5$	NG	NG	-	-
	Intestine	$1.0 \times 10^9$	$4.8 \times 10^8$	NG	NG	-	-
	Feces	$2.2 \times 10^9$	$6.7 \times 10^7$	NG	NG	-	-
	Meat	$1.0 \times 10^9$	$4.8 \times 10^8$	NG	NG	-	-
Local chicken	Feed	$5.7 \times 10^5$	$1.2 \times 10^4$	NG	NG	-	-
	Intestine	$3.8 \times 10^6$	$2.8 \times 10^4$	NG	NG	-	-
	Feces	$9.4 \times 10^9$	$8.0 \times 10^7$	NG	NG	-	-
	Meat	$6.6 \times 10^6$	$8.0 \times 10^3$	NG	NG	-	-
Cow	Feed	$6.0 \times 10^5$	$6.0 \times 10^5$	NG	NG	-	-
	Intestine	$3.8 \times 10^6$	$8.0 \times 10^3$	NG	NG	-	-
	Feces	$9.4 \times 10^9$	$6.8 \times 10^5$	NG	NG	-	-
	Meat	$6.6 \times 10^6$	$1.2 \times 10^4$	NG	NG	-	-

Note: THB, Total heterotrophic bacteria; TC, Total coliform; TP, Total *Pseudomonas*; TS, Total Staphylococci; NG, No Growth.

Table 2: Presence of pathogenic bacteria found in different samples.

Sample	Type	<i>E. coli</i>	<i>Enterobacter</i>	<i>Citrobacter</i> spp.	<i>Klebsiella</i>	<i>Providencia</i>	<i>Acinetobacter</i> spp.
Farm chicken	Feed	-	+	-	+	+	-
	Intestine	+	-	-	+	-	-
	Feces	+	-	-	+	-	-
	Meat	+	+	+	-	-	-
Local Chicken	Feed	-	-	+	-	-	-
	Intestine	-	+	+	-	-	+
	Feces	+	-	-	-	-	-
	Meat	-	-	+	-	-	-
Cow	Feed	+	-	+	-	-	-
	Intestine	+	+	-	-	-	-
	Feces	+	-	+	-	-	-
	Meat	-	-	+	-	-	-

Table 3: Antibiotic sensitivity pattern of bacteria isolated from cow.

Sample	Type	Isolated Bacteria	AMP-10	GN-10	AK-10	AX-10	CTX-30	IMP-10	C-30	TE-30
Cow	Feed	<i>E. coli</i>	R	S	I	S	S	R	S	S
		<i>Citrobacter</i>	R	S	S	I	I	R	I	S
		<i>Citrobacter</i>	R	S	S	R	I	R	S	S
		<i>Citrobacter</i>	R	S	S	R	I	R	R	S
		Resistance (%)	100	0	0	50	0	100	25	0
	Intestine	<i>Enterobacter</i>	R	S	I	R	R	R	R	S
		<i>Enterobacter</i>	R	R	I	R	R	R	I	S
		<i>E. coli</i>	R	S	S	R	S	R	I	S
		<i>Enterobacter</i>	R	I	R	R	R	R	I	S
		Resistance (%)	100	25	25	100	75	100	25	0
	Feces	<i>Citrobacter</i>	R	S	I	I	S	R	I	S
		<i>Citrobacter</i>	R	S	I	R	I	R	I	S
		<i>Citrobacter</i>	R	S	S	R	I	R	I	S
		<i>E. coli</i>	R	S	I	R	R	R	I	S
		Resistance (%)	100	0	0	75	25	100	0	0
	Meat	<i>Citrobacter</i>	R	S	I	I	I	R	I	S
		<i>Citrobacter</i>	R	S	S	R	R	R	S	S
		<i>Citrobacter</i>	R	S	S	R	R	R	I	S
		<i>Citrobacter</i>	R	S	I	R	I	R	R	S
		Resistance (%)	100	0	0	75	50	100	25	0

Table 4: Antibiotic sensitivity pattern of bacteria isolated from local chicken.

Sample	Type	Isolated Bacteria	AMP-10	GN-10	AK-10	AX-10	CTX-30	IMP-10	C-30	TE-30
Local Chicken	Feed	<i>Citrobacter</i>	R	S	I	S	S	R	S	R
		<i>Citrobacter</i>	R	R	R	I	I	R	S	R
		<i>Citrobacter</i>	R	R	R	R	R	R	I	S
		Resistance (%)	100	67	67	33	33	100	0	67
	Intestine	<i>Citrobacter</i>	R	S	I	R	R	R	R	S
		<i>Citrobacter</i>	R	S	I	R	R	R	R	S
		<i>Acinetobacter</i>	R	R	S	R	R	R	R	R
		Resistance (%)	100	33	0	100	100	100	100	33
	Feces	<i>E. coli</i>	R	R	R	R	S	R	R	R
		<i>E. coli</i>	R	R	R	R	S	R	R	R
		Resistance (%)	100	100	100	100	0	100	100	100
	Meat	<i>Citrobacter</i>	R	R	R	R	R	R	R	R
		Resistance (%)	100	100	100	100	100	100	100	100

Table 5: Antibiotic sensitivity patterns of bacteria isolated from farm chicken.

Sample	Type	Isolated Bacteria	AMP-10	GN-10	AK-10	AX-10	CTX-30	IMP-10	C-30	TE-30
Farm chicken	Feed	<i>Klebsiella</i>	R	S	S	R	S	R	S	S
		<i>Klebsiella</i>	R	S	S	R	S	R	S	S
		<i>Enterobacter</i>	R	S	R	R	S	R	S	S
		<i>Klebsiella</i>	R	S	R	R	S	R	R	R
		<i>Providencia</i>	R	S	S	R	S	R	S	S
		Resistance (%)	100	0	40	100	0	100	20	20
	Intestine	<i>E. coli</i>	R	I	I	R	S	R	R	R
		<i>Klebsiella</i>	R	R	S	R	S	R	R	R
		<i>E. coli</i>	R	R	I	R	S	R	R	I
		<i>Klebsiella</i>	R	S	S	R	S	R	R	R
		Resistance (%)	100	50	0	100	0	100	100	75
	Feces	<i>E. coli</i>	R	S	I	R	S	R	R	R
		<i>E. coli</i>	R	S	S	R	S	R	S	R
		<i>Klebsiella</i> spp.	R	S	S	R	S	R	S	R
		<i>E. coli</i>	R	R	S	R	S	R	S	R
		Resistance (%)	100	25	0	100	0	100	25	100
	Meat	<i>E. coli</i>	R	S	I	R	S	R	S	R
		<i>Citrobacter</i> spp.	R	S	S	S	S	R	S	S
		<i>Enterobacter</i>	R	S	I	R	S	R	S	R
		<i>Enterobacter</i>	R	S	R	R	S	R	R	R
<i>E. coli</i>		R	S	I	R	S	R	R	S	
Resistance (%)		100	0	20	80	0	100	40	60	

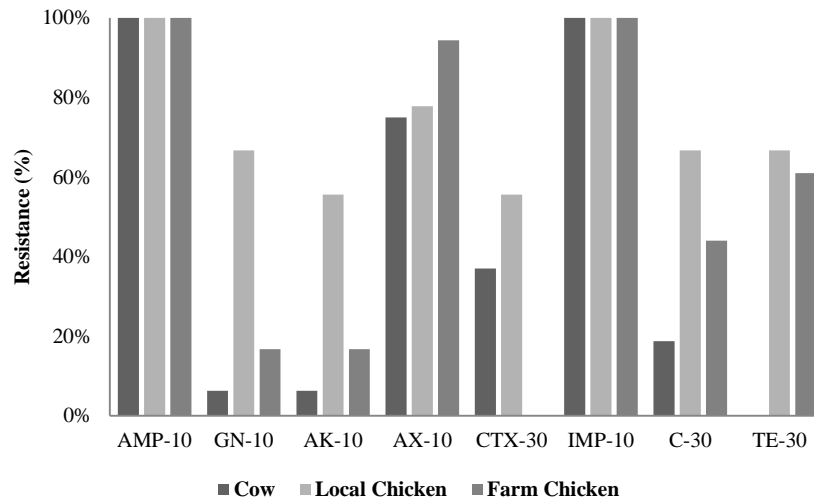


Figure 1: Antibiotic resistance (%) in different types of sample.

The value for each bar indicates the percentage of isolates showing resistance against respective antibiotic.

## DISCUSSION

Antimicrobials have been widely used in food animals for growth promotion since the 1950s (14). Antimicrobial resistance emerges in animal production settings and frequently spreads to humans through the food chain and direct contact (15). Although food animals are sources of antimicrobial resistance, there is little evidence that antimicrobial resistance originates from food animals (16). For this concern, this study was aimed to determine the resistance pattern of bacteria isolated from feed, intestinal contents, feces and meats of local chicken, cow and farm chicken samples.

Incidence of *E. coli* and *Citrobacter* were found in feed, *E. coli*, *Enterobacter* and *Citrobacter* were detected in intestine and feces and *Citrobacter* spp. in meat samples collected from cows. In local chicken samples, *Citrobacter* spp. were found to be present in feed, *E. coli* and *Citrobacter* in intestine and feces and only *Citrobacter* spp. in meat samples. These results indicate that *Citrobacter* spp. was found to be present as common pathogen in cow and local chicken feed, intestine and feces. In addition, it was revealed that cow and local chicken meat could be notable source of contamination by *Citrobacter* spp.

In farm chicken samples *Klebsiella*, *Enterobacter* and *Providencia* were found to be present in feed, *E. coli* and *Klebsiella* in intestine and feces and *E. coli*, *Citrobacter* and *Enterobacter* in meat. These results demonstrate that *Klebsiella* spp. were common pathogen isolated from farm chicken feed, intestine and feces. It was also evident that farm chicken meat can act as source of contamination with *E. coli*, *Citrobacter* spp. and *Enterobacter* spp. All meat

samples were contaminated by some pathogenic bacteria and all of them showed resistance against AMP-10 and IMP-10. However, most of the isolates were sensitive to GN-10, AK-10 and C-30. These results indicate that Gentamicin GN-10, AK-10 and C-30 could still be useful as antibiotics for treatment of poultry, farm grown animals and people infected from these sources. Further more detail studies will be required to address this issue.

According to another report, if an antimicrobial-resistant strain is isolated from a farm, a number of animals in that farm could also harbor that antimicrobial-resistant strain (17). A number of antimicrobial resistant strains could exist in the farm environment, which could influence antimicrobial therapy for both humans and animals (18). In order to reduce the risks associated with the transfer of antibiotic resistant traits from food-producing animals to humans, the usage of antibiotics in veterinary therapy and prevention of bacterial infection in food-producing animals should be minimized.

The considerable detrimental effect of resistant bacteria on the food chain opens the door to the investigation into people exposed to livestock and farms are dangerously vulnerable to infections instigated by antibiotic resistant bacteria existing in the surroundings. A previous research in Thailand (19) demonstrated that 75% of healthy adult food factory workers among 544 were tested positive for a specified resistant bacterium.

Besides, an investigation in Japan carried out on the prevalence of resistance pathogens present in retail meat and food animal feces revealed the isolation of MRSA from 3% of meat samples (9 of 300) and the isolates of *S. aureus* demonstrated the highest resistance towards ampicillin and tetracycline. Additionally, two chicken samples were resistant to ciprofloxacin. The culmination of this research indicated that the origin of the resistant pathogen

could have been the chicken meat (15). Other similar studies infer that the contamination of meat products can occur by resistant *E. coli* at the time of slaughter, and because of having the potential, it can easily make its way to the food chain even though the cattle are administered the antimicrobial growth promoter (AGP). For this reason, the probability of a close connection between livestock and human Salmonellosis across the food chain cannot be ignored (20). Moreover, a ban on the administration of the growth promoting antibiotics appeared to play a role in declining the prevalence of some resistant bacteria in food animals (21).

The impact of antibiotic resistant bacteria remains after the slaughtered livestock are processed into meat and meat products. A study in China revealed that 210 samples from a large scale swine farm carry MDR bacteria posing the potential possibility to be transmitted to humans (22). The results of a Romania investigation (23) exhibited that 23% of 144 chicken carcasses carried MDR *E. coli* and *Salmonella* strains. Similarly, some other research work demonstrated the prevalence of antibiotic resistant pathogens in retail raw poultry in China (24), Italy (25), Nigeria (26), United Kingdom (27) and Vietnam (28). The cited studies identify the raw and processed meat products as a major source of resistant pathogens, and most importantly, the transmission of antibiotic resistant bacteria occurs in the food chain through livestock and their feed (29).

## CONCLUSIONS

In this study microbial load in cow, farm chicken and local chicken samples collected from the local shop demonstrated high load of THB and TC. Samples were frequently found to be contaminated by *E. coli*, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. Only 2 out of 12 samples and 2 out of 43 isolates showed the presence of *Providencia* spp. and *Acinetobacter* spp. Antibiotic resistance pattern was higher amongst the pathogens isolated from chicken samples. All 43 isolates of this study showed 100% resistance against AMP-10 and IMP-10 and 19 of 43 isolates showed 33 to 80% resistance and remaining 22 of 43 isolates showed 100% resistance against AX-10. This is a matter of concern for local shops selling cow and chicken in Dhaka city, Bangladesh. Necessary steps should be taken to monitor and restrict the use of antibiotics in feeds. It will be also necessary to create awareness and stewardship among the livestock farmers, businessmen and general public to reduce the propagation and spread of antibiotic resistance bacteria. An elaborate study will be required to determine the source of pathogen and antibiotic resistant trait and suggest necessary steps to be taken for appropriate intervention.

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