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ORIGINAL ARTICLE

Antibiogram of *E. coli* and *Salmonella* spp. isolated from chicken meat and frozen milk in Barishal city, Bangladesh

M. A. Rahman, M. S. Ahmed

Department of Medicine, Surgery and Obstetrics, Faculty of Animal science and Veterinary Medicine, Patuakhali Science and Technology University, Babugonj, Barishal, Bangladesh

Abstract

Background: Food safety is now a global issue especially in developing countries like Bangladesh. Foodborne diseases are leading causes of illness and death in man, animals and birds in the world. Antibiotic resistant *Salmonella* and *E. coli* may transfer these resistances to humans through consuming chicken meat and frozen milk available in the market. There is no study on the microbiological load in chicken meat and frozen milk marketed in Barishal city. Therefore, the present study was designed to assess the contamination of *E. coli* and *Salmonella* spp. in chicken meat and frozen milk along with their antimicrobial resistance pattern in Barishal city.

Methods: A total of 40 samples including frozen milk (n=20) and chicken meat (n=20) were aseptically collected between July 2020 and June 2021 from a renowned shop and open retail market of the municipal area in Barishal city. The isolation and identification of *E. coli* and *Salmonella* spp. were done by conventional techniques. The antimicrobial profile was evaluated through a disk diffusion method.

Results: The overall detection of *E. coli* and *Salmonella* spp. were 62.5% and 32.5% in milk and chicken meat specimens. No salmonella was detected in milk samples whereas *Salmonella* spp. was detected only in 65% of chicken meat samples. An overall 100% of the isolates were multi-drug resistant (MDR). Both *E. coli* and *Salmonella* spp. isolated from chicken meat and milk were highly sensitive to amikacin (71%-100%) followed by gentamicin (64%-87%), sulpha-trimethoprim (13%-67%); and highly resistant (100%) to oxytetracycline, amoxicillin, and ciprofloxacin.

Conclusions: Chicken meat and milk should be cooked or heated thoroughly before consumption. Amikacin, gentamicin, and sulfa-trimethoprim should be the drugs of choice for the treatment of salmonellosis and colibacillosis in dairy and poultry in the study area. Hygienic and sanitary measures should be taken in all aspects from the farm to fork.

Keywords: Amikacin, Gentamicin, Multi-drug resistance, Detection

*Correspondence: email: anisbgvc@yahoo.com

Introduction

Food of animal origin like chicken meat and milk are rich in protein which are very essential for body growth and development. Animal origin foods act as a medium of transmission of microorganisms to humans as consumers. Foods, even safely cooked and ready-to-eat foods, can become cross-contaminated with pathogens causing considerable public health burden and challenge due to unhygienic processing, handling and production. Foodborne diseases (FBD) causes nausea, abdominal cramps, vomiting, diarrhea, or fever and death in serious illnesses especially in infants, children, elderly and immune-compromised persons (Rahman *et al.,* 2017). Among the foodborne bacterial pathogens *Salmonella* spp. and *E. coli* are the most prevalent having zoonotic significance worldwide causing diarrheal diseases which are endemic in Bangladesh and 20,000 children less than 5 years old die of diarrheal diseases in Bangladesh (Huda *et al.,* 2012). About 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths occur due to salmonellosis (Momtaz *et al*., 2018). Castro-Vargas *et al*. (2020) reported that 93 million cases of gastroenteritis and 155,000 deaths occur worldwide. A wide range of antimicrobial drugs are used in livestock and poultry for treatment, herd and flock health management. Antibiotic resistances are increased due to improper dosages, neglecting withdrawal period and indiscriminate use of antimicrobials causing adverse effect on public health exhibiting reduced susceptibility to common antibiotics through a variety of mechanisms like mutations, conjugations, transformation etc. (Zishiri *et al*., 2016). People especially of Barishal city are inclined to buy processed chicken meat and raw milk from the open market as well as frozen chicken meat and milk (with/without pasteurized) from super shops to save time and food processing hazard. The microbiological safety of this frozen chicken meat, milk or raw meat is questionable in the context of public health hazards because some pathogenic strains of *E. coli* are resistant to high heat and to low temperature/ cold shock (Parvin *et al.,* 2020). Modern food production (industrialization) facilitates the emergence and spread of resistance through the intensive use of antimicrobial agents and international trade of both animals and food products. The main route of transmission between food animals and humans is via food products, although other modes of transmission, such as direct contact and through the environment, also occur. Resistance can spread as resistant pathogens or via transferable genes in different commensal bacteria. Chicken meat and milk are supplied from different corners of the Barishal division to the metropolitan area at a high price and to meet protein demand of the city dwellers. Therefore microbiological investigations on these foods sold in open market and super shops will give a general picture of quality control and hygienic measures in poultry and dairy farms situated in South Bengal region.

Materials and methods

Study area and duration

The super shops and open market were selected randomly from Barishal metropolitan city, Barishal. The study was conducted between July 2020 and June 2021.

Sample collection and preparation

A total of 40 samples (20 frozen milk samples from four companies and other local dairy farms; and 20 chicken meat samples from open markets like Chawmatha, Nothullabad, Battola, Chakbazar and Bangla bazar in Barishal city) were collected. About 10-20 grams of raw chicken meat in a sterilized zipper bag; and 10 ml of frozen milk (supplied from different local dairy farm) in sterilized test tube from each sample (250 ml or 500 ml frozen plastic bag) were collected aseptically and transported immediately using an icebox to the Department of Medicine, Surgery and Obstetrics laboratory for bacteriological investigation. Ten to twenty grams of chicken meat were mixed with 10 ml of peptone (0.1%) water then homogenized suspension was prepared using sterilized pestle and mortar in laminar air flow.

Isolation and identification of *Salmonella* spp. and *E. coli*

Isolation and identification of *E. coli* and *Salmonella* spp. were done by conventional methods. Different bacteriological culture media (Nutrient broth, Nutrient agar, Eosin-methylene blue agar, Salmonella-shigella agar, Xylose-lysine Deoxycholate agar, MacConkey agar, Muller-Hinton agar, Triple sugar Iron phosphate agar) have been prepared aseptically under laminar air flow according to instruction of manufacturer's company (Hi-media, India) before starting of bacterial culture.

The homogenized meat suspension (4 to 5 ml) was inoculated into nutrient broths (5 ml/ test tube) followed by nutrient agar at 37^0C for 24hours in each case. The suspected colony were then inoculated through inoculating loop onto Salmonella-shigella (SS) agar and Xylose-lysine Deoxycholate (XLD) agar for salmonella; as well as onto Eosin-methylene blue (EMB) agar for *E. coli* which were then incubated at 37^0C for 24±2 hours for isolation. Culture positive samples were subcultured several times to obtain a pure colony. Gram staining (40X, under microscope) and biochemical tests were performed (by TSI agar) through inoculating of suspected isolated bacterial colony into each test tubes containing 5 ml of TSI (triple sugar iron phosphate agar) agar media and incubated for 24 hours at 37^0C (Cheesbrough, 1985).

About 1 ml of milk from the test tube was transferred into nutrient broth (5ml) and incubated at 37^0C for 24 hours. Then from turbid nutrient broth it was inoculated through an inoculating loop into different selective agar like SS agar, XLD agar for *Salmonella* spp.; and EMB agar and MacConkey agar for *E. coli* isolation. The inoculated media was incubated at 37^0 C for 24±2 hours in separate packs. Culture positive samples were subcultured several times in selective media to be pure culture as above mentioned procedures (Cheessbrough, 1985).

Antimicrobial susceptibility test

Antimicrobial susceptibility of *E. coli* and *Salmonella* spp., was performed by the disc diffusion method according to the guidelines of the CLS1 (2018). The broth culture method equivalent to 0.5 McFarland solution was used for AST in both *E. coli* and *Salmonella* spp. before inoculating the culture onto Mueller-Hinton agar. Ten commercially available antibiotics (Oxoid™, UK) e.g., oxytetracycline (30µg), ciprofloxacin (5µg), gentamicin (10µg), ceftriaxone (30µg), azithromycin (15µg), sulfonamide-trimethoprim (25µg), neomycin (10µg), amoxicillin (10µg), oxacillin (1µg) and amikacin (30µg) were used for antibiogram study.

Results

Detection rate

The *E. coli* and *Salmonella* spp. were identified in chicken meat and frozen milk. The overall detection rate of *E. coli* and *Salmonella* spp. were 62.5% and 32.5%, respectively. No Salmonella was detected in milk samples. About 65% of chicken meat samples contained *Salmonella* spp.. The *E. coli* was detected in 75% and 50% of Chicken meat and frozen milk samples, respectively. Among the isolated bacteria *E. coli* was more frequently detected than that of *Salmonella* spp. in chicken meat samples (Table1).

Cultural, staining and biochemical characteristics

E. coli and *Salmonella* spp. produced turbid growth on nutrient broth and smooth white to grayish white colony on nutrient agar with peculiar fetid odor. *E. coli* produced metallic sheen colonies on EMB agar (Figure 1a) and *Salmonella* produced black centered colonies on SS agar (Figure 1d) and XLD agar (Figure 2a). On Gram staining both *E. coli* and *Salmonella* spp. were found Gram negative, rod shaped; and arranged as single, paired or chain form (Figure 1b and Figure 2b). On TSI agar *E. coli* produced acid and gas on slant by fermentation of dextrose, sucrose and lactose evidenced by yellow colored agar (Figure 1c) whereas salmonella produced hydrogen sulfide evidenced by blackening of agar (Figure 2c).

Table 1: Prevalence of *E. coli* and *Salmonella* spp. in chicken meat and milk samples

Sample	No. of sample	No. of Positive case (Prevalence in %)	
		E. coli	*Salmonella* spp.
Chicken meat	20	15 (75.0)	13 (65.0)
Frozen milk	20	10 (5.0)	0 (0)
Total	40	25 (62.5)	13 (32.5)

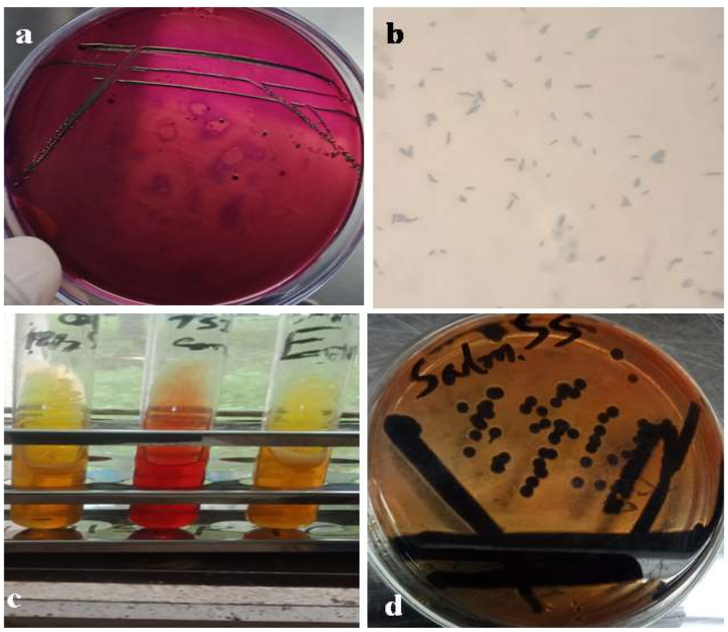

Figure 1 a) Growth of *E. coli* on EMB agar showing metallic sheen single colony, b) Gram's stain of *E.coli* showing gram negative rod shaped arranged in single, pair or chain form (40x), c) Test of *E. coli* in TSI agar showing production of acid (yellow color) and gas, control in the middle, d) Growth of *Salmonella* sp. on SS agar showing black centered colony.

E. coli and Salmonella spp. isolated from chicken meat and frozen milk

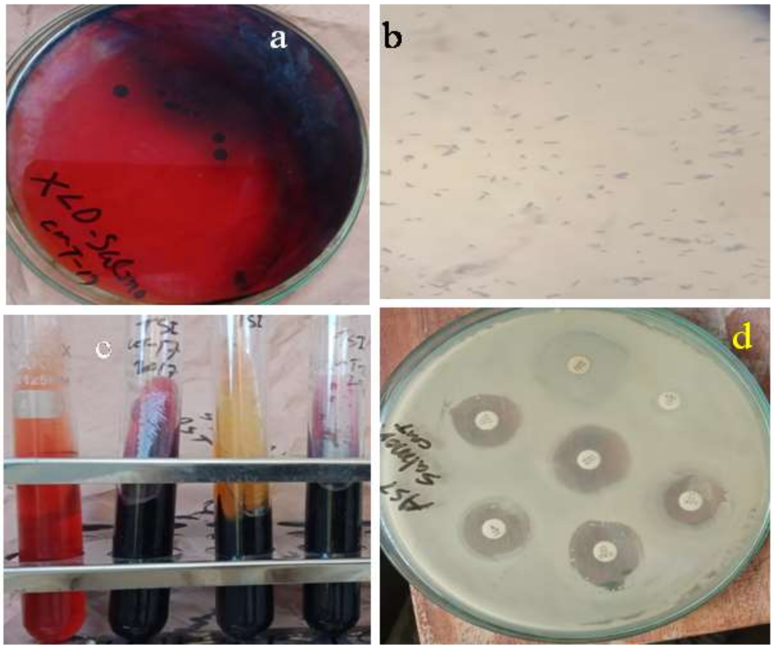

Figure 2 a) Growth of *Salmonella* spp. on XLD agar showing Black centered colony, b) *Salmonella sp.* on Gram's stain showing gram negative small rod shaped arranged in single or pair form (40x), c) *Salmonella* spp. on TSI agar showing production of hydrogen sulfide (black color agar) and gas, control in the left, d) Antimicrobial susceptibility test of *Salmonella* spp. showing resistant to oxytetracycline and amoxicillin; sensitive to Amikacin, ceftriaxone, azithromycin, streptomycin and sulfa trimethoprim

Table 2: The prevalence of MDR in *E. coli* and *Salmonella* spp., in chicken meat and frozen meat isolates

	Chicken meat	Frozen Milk	Total
No. of *E. coli* isolates	14	3	17
Multi-drug resistance (%)	14 (100)	2 (66.67)	16 (94.12)
No. of *Salmonella* spp. isolates	13	-	13
Multi-drug resistance (%)	13 (100)	-	13 (100)

Table 3. Antibiogram study of *E. coli* and *Salmonella* spp. isolated from chicken meat and frozen milk

Antibiotic used	*E. coli*				*Salmonella* spp.	
	Chicken meat (n=14)		Frozen milk (n=3)		Chicken meat (n=13)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin	04 (28.6)	10 (71.4)	0 (0)	03 (100)	0 (0)	13 (100)
Streptomycin	09 (64.3)	05 (35.7)	01 (33.33)	02 (66.67)	11 (84.62)	02 (15.38)
Gentamicin	05 (35.7)	09 (64.3)	0 (0)	03 (100)	02 (15.38)	11 (84.62)
Azithromycin	09 (64.3)	05 (35.7)	02 (66.67)	01 (33.33)	10 (76.93)	03 (23.07)
Oxytetracycline	14 (100)	0 (0)	03 (100)	0 (0)	13 (100)	0 (0)
Amoxicillin	14 (100)	0 (0)	03 (100)	0 (0)	13 (100)	0 (0)
Oxacillin	14 (100)	0 (0)	03 (100)	0 (0)	13 (100)	0 (0)
Sulfamethoxazole - trimethoprim	13 (92.86)	01 (7.14)	01 (33.33)	02 (66.67)	11 (84.62)	02 (15.38)
Ceftriaxone	08 (57.3)	06 (42.9)	03 (100)	0 (0)	09 (69.24)	04 (30.76)
Ciprofloxacin	13 (92.86)	01 (7.14)	02 (66.67)	01 (33.33)	13 (100)	0 (0)

R= Resistant; S= Sensitive

Antibiogram study

The result of an antibiogram study of 10 commonly used antimicrobials is shown in Tables 2, 3 and Figure 2d. About 100% of *E. coli* and *Salmonella* spp. isolated from chicken meat were multidrug resistant (MDR). *E. coli* isolated from frozen milk were 66.67 % MDR. Both *E. coli* and *Salmonella* spp. isolated from chicken meat were highly sensitive to amikacin (71%-100%) followed by gentamicin (64%-87%), sulpha-trimethoprim (67%); and highly resistant (100%) to oxytetracycline, amoxicillin and ciprofloxacin. The *E. coli* isolated from frozen milk were sensitive to amikacin, gentamicin followed by sulpha-trimethoprim and streptomycin.

Discussion

We detected *E. coli* and *Salmonella* spp. in chicken meat and frozen milk samples. All isolates were multidrug resistant. Based on the results we recommended consuming chicken meat and milk after proper cooking. Moreover, hygienic and sanitary measures in all aspects from farm to fork are also needed.

The overall detection rate of *E. coli* was 62.5% of which 50% in milk and 75% in chicken meat specimens. Similarly, Parvin *et al*., (2020) also reported 76% prevalence of *E. coli* in frozen chicken meat. Rahman *et al*., (2017) reported 30% *E. coli* in milk and 49% in chicken meat from other parts of Bangladesh. Two samples collected from pasteurized frozen milk of one brand were contaminated with *E. coli*. Similarly, Kumar *et al*., (2020) found 33.33% pasteurized milk contaminated with *E. coli* in India. The pathogenicity of *E. coli* isolated in this study was not tested. It is known that some *E. coli* are heat resistant and might be contaminated due to unhygienic handling or poor storage after pasteurization (Kumar *et al*., 2020) and causes serious health hazards in humans (Parvin *et al*., 2020). *E. coli* isolated from chicken meat were highly resistant (100%) to oxytetracycline, amoxicillin, oxacillin and sulpha-trimethoprim (93%). Similar observations were reported by

Parvin *et al*., (2020) where *E. coli* were highly resistant to oxytetracycline (93%), amoxicillin (92%) and sulpha-trimethoprim (88%); and also parallelly observed by Rahman *et al*., (2020).

In this study, *E. coli* isolated from chicken meat were 100% MDR which is in agreement with the previous reports (Parvin *et al*., 2020; Alvarez-Fernandez *et al*., 2013; Rahman *et al*., 2017). On the other hand, 67% *E. coli* of frozen milk were MDR which is higher than that of Rahman *et al*., (2017). Indiscriminate use with improper selection and dose of antibiotics and incomplete duration of antibiotic course at farm level may be responsible for MDR in *E. coli*. On the other hand, the overall detection rate of *Salmonella* spp. was 32.5% of which none in milk but 65% in chicken meat. Similar finding was reported by Rahman *et al*., (2018a) where they detected 57% salmonella in chicken meat. Other authors reported the occurrence of *Salmonella* spp. in 7% to 40% of the chicken meat samples (Rabby *et al*., 2021; Amin *et al*., 2015; Aslam *et al*., 2012) in Bangladesh. Previous studies reported variable (23.7%-65%) prevalence of *Salmonella* spp. in chicken meat from different countries (Kaushik *et al*., 2014; Ruban and Fairoze, 2011; Bhandari *et al*., 2013); Zishiri *et al*., 2016; Zhu *et al*., 2017). Similarly, very low prevalence of *Salmonella* spp. in milk (3.63%) were reported in Iran (Tajbakhsh *et al*., 2012). The variation in the prevalence might be due to differences in sample type, test procedure and hygienic maintenance of the food processing. Contamination occurs due to unhygienic slaughtering, handling, cutting, processing and storage (Momtaz *et al*., 2018). Milk were found free of *Salmonella* spp. that might be due to pasteurized milk marketed by different companies or not mixing of external water and using sterile utensils during collecting and packaging of milk (Munsi *et al*., 2015).

An overall 100% isolates of *Salmonella* spp. in chicken meat were found to be multidrug resistant (MDR) which is in line with Rahman *et al*., (2018a). Low prevalence of MDR *Salmonella* spp. varying from 16.67% to 23.5% were reported by other authors (Al-salauddin *et al*., 2015; Dallal *et al*., 2009). This indicates MDR *Salmonella* spp. is increasing with the time due to indiscriminate use of several antibiotics at a time in poultry farms to check their morbidity and mortality in Bangladesh. The inherent gene responsible for MDR may transfer to consumers through chicken meat handling (or undercooked) and may result in serious public health threat (Hossain *et al*., 2021; Rahman *et al*., 2018a). Rational use of these drugs may prevent MDR in salmonella in future.

About 100% *Salmonella* spp. in chicken meat were resistant to oxytetracycline, amoxicillin and oxacillin followed by streptomycin, sulpha-trimethoprim and ceftriaxone. Various drug resistance patterns of *Salmonella* were reported by Hossian *et al*., (2021). Amikacin and gentamicin were found the most sensitive in this study and could be used as the best choice of drugs in the treatment of salmonellosis and colibacillosis of poultry as well as sulpha-trimethoprim and streptomycin in colibacillosis in dairy cattle. Similar reports were also published by other authors (Rahman *et al*., 2018b; Parvej *et al*. 2016; Al-salauddin *et al*., 2015).

Conclusion

The detection of *E. coli* and *Salmonella* spp. in chicken meat and frozen milk indicates these foods were contaminated due to improper handling, processing, storage, poor personal hygiene and sanitation. These bacteria are found to be resistant to drugs commonly used in humans like oxytetracycline. MDR is a global issue and resistant bacteria can be transferred to humans through contaminated foods which may pose serious public health hazards. We recommend amikacin, gentamicin and sulfa-trimethoprim drugs in the treatment of salmonellosis and colibacillosis in dairy and poultry in the study area. Chicken meat and milk should be cooked or heated thoroughly before consumption. Hygienic and sanitary measures should be taken in all aspects from farm to fork. Government and livestock agencies should take the responsibility for monitoring and surveillance in relation to the prescription which should be written by the registered veterinarian to prevent misuse of antibiotics.

Competing Interest

The authors declare that they have no competing interests.

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