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ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM STUDY OF *SALMONELLA* ISOLATED FROM APPARENTLY HEALTHY JAPANESE QUAIL

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ABSTRACT

Salmonellosis is a major public health problem around the world affecting both animals and humans caused by various species of *Salmonella*. The present study was aimed at isolation, identification and antibiogram profiling of *Salmonella* spp. isolated from cloacal swabs of apparently healthy Japanese quail of selected live bird markets at Dinajpur Sadar Upazila under Dinajpur district of Bangladesh. The isolates were identified based on their morphological, cultural and biochemical characteristics and motility test. While the antibiogram profiling of the *Salmonella* isolates were performed with standard disc diffusion methods. Among the collected samples (n=53), 16.98% samples (n=9/53) were found positive for *Salmonella* spp. Interestingly, all isolates were found to be motile while tested with Motility Indole Urease (MIU) test. The antibiogram study revealed that all the isolates were completely (100%) resistant to Amoxicillin, Gentamicin, Erythromycin, Tetracycline and Ceftriaxone, whereas the sensitivity rate of the isolates to Ciprofloxacin, Amikacin and Streptomycin were 77.8%, 55.6% and 33.3%, respectively. While, among the resistant *Salmonella* isolates, all were multidrug resistant (MDR). Therefore, findings of the present research work suggested that presence of multidrug resistant *Salmonella* spp. in quails could be a potential threat for public health.

1. INTRODUCTION

Poultry industry is an important sub-sector of livestock production in Bangladesh that plays a crucial role in economic growth and simultaneously creates numerous employment opportunities. As a fundamental part of animal production in Bangladesh, the industry is committed to supplying nation with a cheap source of good quality nutritious animal protein in terms of meat and eggs. Approximately 25% of the protein consumed in Bangladesh originates from poultry. Despite this data, Bangladesh is still one of the lowest poultry meat consuming countries in the world. Here, per capita meat consumption is only 1.2 kg per person per year and per capita egg consumption is also about 32 eggs per person per year. With increasing incomes, the demand for meat, especially the cheaper option of poultry meat, and eggs is set to rise. More importantly, quail becomes a promising poultry species in Bangladesh (Redoy et al., 2017).

Quail (*Coturnix japonica*) is a new addition in the poultry industry of Bangladesh. The number of quail farm is increasing day by day due to its easy management, faster growth rate, early sexual maturity, highly palatable meat, high nutritional value of meat and egg, high rate of egg production and requirement of minimum floor space for quail. Quails are more resistant to infectious diseases than chickens although few infectious diseases are encountered in quails. The advancement of quail production is being hampered by some management factors, fatal infectious, non-infectious and parasitic diseases (Barnes, 1987).

Salmonellosis is an important bacterial disease that causes serious economic loss manifested by reduced egg production and mortality of poultry (Khan et al., 2005). Although Japanese quails are comparatively more resistant to infectious diseases than chickens, like salmonellosis, coccidiosis, infectious coryza, enteric diarrhea and pneumonia (Rahman et al., 2016). Each year, salmonellosis may be responsible for gastroenteritis and deaths in both developed and developing countries (Kennedy et al., 2004; Majowicz et al., 2010; Rothrock et al., 2015). There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths found annually worldwide due to *Salmonella* (Bhunua, 2018). Center for Disease Control and Prevention (CDC) reported that in USA, *S. infantis*, *S. newport* and *S. hadar* are found in live poultry and 80% of salmonellosis affected patients contact live poultry before ill (Shu et al., 2015). Clinical patterns of salmonellosis cases are enteric fever, gastroenteritis, bacteremia, and chronic carrier state (Shu et al., 2015).

In most cases, standard of dose and duration of antibiotic treatment are not followed. As a result, antibiotic resistant bacteria are emerging rapidly, which may transmit to humans through food which are originated from poultry. Most of the people affected food-borne infections by multidrug resistant (MDR) *Salmonella* by consumption of contaminated foods of animal origin (Angulo et al., 2000).

The present study was designed to isolate and identify *Salmonella* spp. from apparently healthy Japanese quail (*Coturnix japonica*) and to determine antibiotic resistance patterns of identified isolates.

2. MATERIALS AND METHODS

To complete this study, a total of 53 cloacal swab samples were collected from apparently healthy Japanese quail from several retail quail selling shops in Sadar Upazila of Dinajpur district of Bangladesh. Following collection, the swabs were inserted into the supplied sterile container with phosphate buffered saline (PBS) and were carried in an ice box to the Bacteriology laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur and processed for the isolation and characterization of *Salmonella* spp. All the samples were collected aseptically.

2.1 Isolation and Identification of *Salmonella* spp.

The processed samples were primarily cultured into Nutrient agar, Nutrient broth, Bacto selenite broth then secondarily cultured into MacConkey agar, Eosin methylene blue agar and then cultured on selective media such as Salmonella-Shigella agar, Brilliant green agar, Xylose-Lysine Deoxycholate agar. Petri dishes were incubated in the bacteriological incubator at 37^o C for overnight for bacterial growth. *Salmonellae* were identified and confirmed according to colony

characteristics found on the agar plate (Carter GR. 1979).

Gram's staining method was followed to study the morphological and staining characteristics of the isolated bacteria and to provide information about the presumptive bacterial identification as per recommendation of Merchant and Packer (1967)

Isolated organisms with supporting growth characteristics of *Salmonellae* on various culture media were subjected to different biochemical tests such as Methyl Red (MR) test, Voges-Proskauer (VP) test, Indole reaction, Sugar fermentation test, Motility Indole Urease (MIU) test and Triple Sugar Iron (TSI) agar slant reaction according to the standard procedures described by Merchant and Packer (1967).

2.2 Antibiotic sensitivity test

It was performed according to the procedure of Kirby-bauer disk diffusion method. Drug resistance patterns of the isolated *Salmonella* spp. were detected by disc diffusion method by using commonly used 10 antibiotics on freshly prepared Mueller-Hinton agar (Oxoid) plate. Isolates were classified as susceptible, intermediate and resistant categories based on the standard guidelines of Clinical and Laboratory Standards Institute (CLSI, 2018).

3. RESULTS

A total of 53 samples were isolated from cloacal swab of apparently healthy Japanese quail from different local markets in Sadar Upazila of Dinajpur district and 9 were found to be positive. To identify *Salmonella* spp. the collected samples were subjected to various bacteriological examinations and antibiotic sensitivity test in the laboratory of the Department of microbiology, HSTU, Dinajpur. After inoculation of cloacal swab sample on Nutrient agar and Nutrient broth, it was observed that maximum growth was found in Nutrient agar produced translucent, opaque, smooth, colonies. On Nutrient broth *Salmonella* spp. isolates produced turbidity.

In different bacteriological culture media, isolated *Salmonella* spp. produced different type of colonies. In MacConkey agar, colonies produced by *Salmonella* spp. were colorless, smooth, transparent, and raised (**Fig-1**). It produced pink color colony on EMB agar. On SS agar, all the isolates produced translucent, black, smooth, small, and round colonies which were positive for *Salmonella* spp. (**Fig-2**) and on XLD agar media, it produced pink color colony with black center (**Fig-3**). *Salmonella* spp. isolates produced pink color colonies against rose pink color background in Brilliant green agar (**Fig-4**). The microscopic examination of Gram's stain revealed Gram-negative, pink colored, short rod-shaped bacteria, arranged in single and paired (**Fig-5**) (**Table-1**).

In biochemical s, it gave negative result in Indole test and Voges- Proskauer test and gave positive results in Methyl Red test, Simmons' citrate agar slant test, and TSI agar slant test. Among 9 isolates were found to be motile characterized by forming the stab line with producing turbidity in the Motility Insole Urease (MIU) test (**Table-2**).

The overall prevalence of *Salmonella* in cloacal swab of apparently healthy Japanese quail was 16.98% (n=9/53). The highest percentage of *Salmonella* (26.7%) isolates were observed at Bahadur bazar and the lowest percentage of *Salmonella* (7.7%) isolates were observed at Labor More in Sadar, Dinajpur (**Table-3,4**).

All 9 positive samples were undergone antimicrobial susceptibility test and 9 positive isolates were susceptible to Azithromycin (11.1%), Streptomycin (33.3%), Ciprofloxacin (77.8%), Ceftriaxone (100%) and Amikacin (55.6%). Isolates (n=9) were less susceptible to Azithromycin (11.1%) and Streptomycin (33.3%). Isolates (n=9) were resistant to Amoxicillin (100%), Gentamicin (100%), Erythromycin (100%), Tetracycline (100%), Doxycycline (77.8%), Azithromycin (66.7%) and Streptomycin (55.6%). Some isolates were intermediate to Azithromycin (22.2%), Streptomycin (11.1%), Ciprofloxacin (22.2%), Amikacin (44.4%) and Doxycycline (22.2%) (**Table-5**) (**Fig-6**)

4. DISCUSSIONS

The present study was focused on the isolation and identification of *Salmonella* spp. based on morphological, cultural, and biochemical properties (Sander *et al.*, 2001; Bacci *et al.*, 2012). For this purpose, cloacal swabs (n=53) from apparently healthy Japanese quail were considered as initial samples from which, n=9/53 were positive for *Salmonella* spp.

The prevalence of *Salmonella* spp. isolated from cloacal swab samples collected from apparently healthy

Japanese quail was compared with the findings of Jahan *et al.*, (2018) and Palanisamy and Bamaiyi (2015). In this study, the prevalence of *Salmonella* spp. was found to be 16.98% (n=9/53), which was higher than the observation of Jahan *et al.*, (2018). In their study, the prevalence of *Salmonella* spp. in quails was 13.33% (n=10/75) while Palanisamy and Bamaiyi (2015) showed that the prevalence of *Salmonella* spp. in the quails was 11.11%. Some authors could not be able to isolate *Salmonella* from quail (McCrea *et al.*, 2006; Dipineto *et al.*, 2014) whereas several others could isolate *Salmonella* from quail (Sander *et al.*, 2001; Bacci *et al.*, 2012; Udhavavel *et al.*, 2016). This variation in prevalence might be due to the variation in management practices and difference in number of sample or due to various areas. Unhygienic management may act as the cause of high prevalence of *Salmonella* spp.

Antimicrobial resistance test was performed by disc diffusion method using 10 different antibiotics. In this study, the isolated *Salmonella* showed 100% resistant to Amoxicillin, Gentamicin, Erythromycin and Tetracycline indicating that the isolates were multidrug resistant and near about resistant to Doxycycline (77.8%), Azithromycin (66.7%) and Streptomycin (55.6%). The resistance of Tetracycline and Erythromycin of this study were compared with the findings of Jahan *et al.* (2018) and Jahan *et al.* (2013) who showed in their study that isolates were 100% resistant to Tetracycline and Erythromycin similar to the present study. In this study, all isolates were susceptible to Azithromycin (11.1%), Streptomycin (33.3%), Amikacin (55.6%), Ciprofloxacin (77.8%) and Ceftriaxone (100%). The susceptibility of *Salmonella* spp. to Ciprofloxacin was compared with the findings of Jahan *et al.* (2018) and Ramya *et al.* (2013). Both studies found 100% susceptibility of isolates to Ciprofloxacin whereas the present study found 77.8% susceptibility. This difference could be due to the increasing rate of inappropriate utilization of antibiotics which favors selection pressure that increased the advantage of maintaining resistance genes in bacteria (Mathew *et al.*, 2007). The antibiotic resistant genes of these isolates may transfer to *Salmonella* that may infect both human and animal hindering their health (Uduak 2015; Wakawa *et al.*, 2015).

5. CONCLUSIONS

In this study, the overall prevalence of *Salmonella* spp. in apparently healthy Japanese quail was 16.98% (n=9/53). The degrees of sensitivity of *Salmonella* spp. with various antibiotics were determined by agar disc diffusion method using 10 different antibiotics. The patterns of drug sensitivity of the isolates were found to be variable. Antibiogram study showed that *Salmonella* spp. isolated from apparently healthy Japanese quail were 100% resistant to Amoxicillin, Gentamicin, Erythromycin and Tetracycline and near about resistant to Doxycycline (77.8%), Azithromycin (66.7%) and Streptomycin (55.6%). All isolates were susceptible to Azithromycin (11.1%), Streptomycin (33.3%), Amikacin (55.6%), Ciprofloxacin (77.8%) and Ceftriaxone (100%). The present study revealed the presence of *Salmonella* in apparently healthy Japanese quail and resistance of *Salmonella* to most antimicrobials except ciprofloxacin, Ceftriaxone and Amikacin. The MDR *Salmonella* spp. in healthy quail may be transferred to the humans and animals. Based on our present findings, recommendations are forwarded such as training programs must be provided on best practice of rearing of quail for farmers and raising the level of awareness of people. Since *Salmonella* is resistant to most common drugs, attention should be taken in selecting antimicrobials in treating *Salmonella* infection both in animals and human being based on antimicrobial susceptibility test.

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Conflict of interests

The authors declare no conflict of interest.

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TABLES

Table 1. Cultural and staining characteristics of *Salmonella* spp. isolated from apparently healthy Japanese quail (Cloacal swab sample).

Isolated organism	Staining Characteristics	Media used	Colony characteristics	Motility Characteristics
<i>Salmonella</i> spp.	Gram negative short rod-shaped pink	Salmonella-Shigella agar	Translucent, smooth, small round black centered colonies	All isolates were motile and produced turbidity throughout the tube
		MacConkey agar	Colorless, smooth, transparent, raised colonies	
		Brilliant green agar	Pale pink color colonies against a red background	
		Xylose Lysine Deoxycholate (XLD) agar	Pink color colony with black center	

	color	Nutrient agar	Translucent, smooth, opaque colonies
		Nutrient broth	Turbidity on whole broth

Table 2. Results of different biochemical tests for the representative isolates.

Isolated organism	IN	MR	VP	SC	TSI	MIU
<i>Salmonella</i> spp.	-	+	-	+	+	+

Legends: + = Positive; - = Negative; IN =Indole; MR =Methyl-Red; VP = Voges Proskauer; SC = Simmons Citrate; TSI = Triple Sugar Iron; MIU = Motility Indole Urease

Table 3. Over all prevalence of *Salmonella* spp. isolated from cloacal swabs

Total number of isolates	Number of positive isolates	Positive Percentage	Number of negative case	Negative Percentage
53	9	16.98%	44	83.02%

Table 4. Prevalence of isolated *Salmonella* spp. from different local markets of Sadar Upazila of Dinajpur district.

Name of the place	No. of sample	No. of positive isolates	Prevalence
Suihari	15	3	20%
Bahadur Bazar	15	4	26.7%
Ramnagor	10	1	10%
Labur More	13	1	7.7%
Total	53	9	16.98%

Table 5. Antimicrobial susceptibility profile of isolated *Salmonella* spp.

Antimicrobial agents	Number of <i>Salmonella</i> isolates		
	S	I	R
Azithromycin	1 (11.1%)	2 (22.2%)	6 (66.7%)
Streptomycin	3 (33.3%)	1 (11.1%)	5 (55.6%)
Ciprofloxacin	7 (77.8%)	2 (22.2%)	0
Amoxicillin	0	0	9 (100%)
Gentamicin	0	0	9 (100%)
Ceftriaxone	9 (100%)	0	0

Erythromycin	0	0	9 (100%)
Tetracycline	0	0	9 (100%)
Amikacin	5 (55.6%)	4 (44.4%)	0
Doxycycline	0	2 (22.2%)	7 (77.8%)

Legends: S=Susceptible; I=Intermediate; R=Resistant

FIGURES

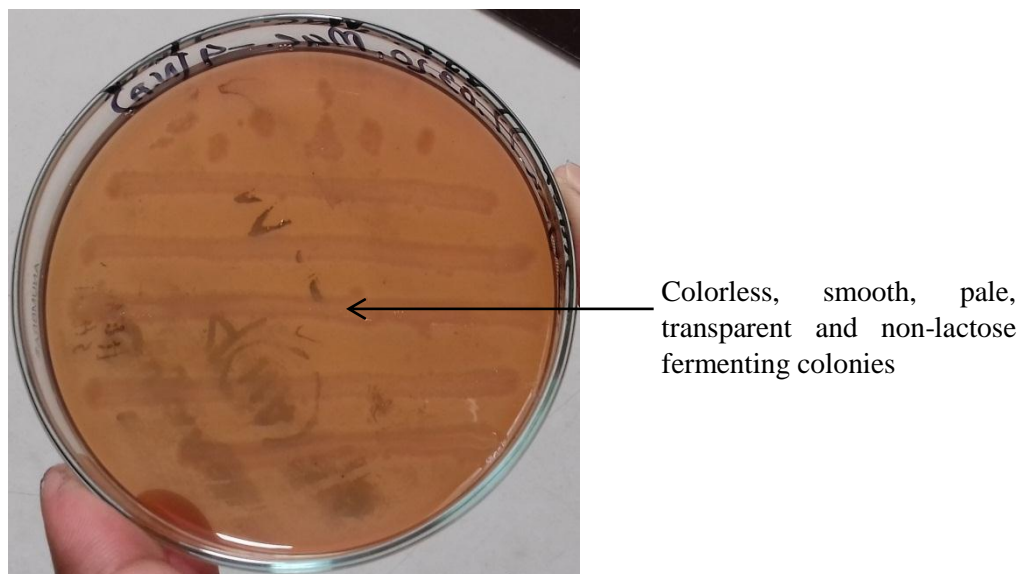
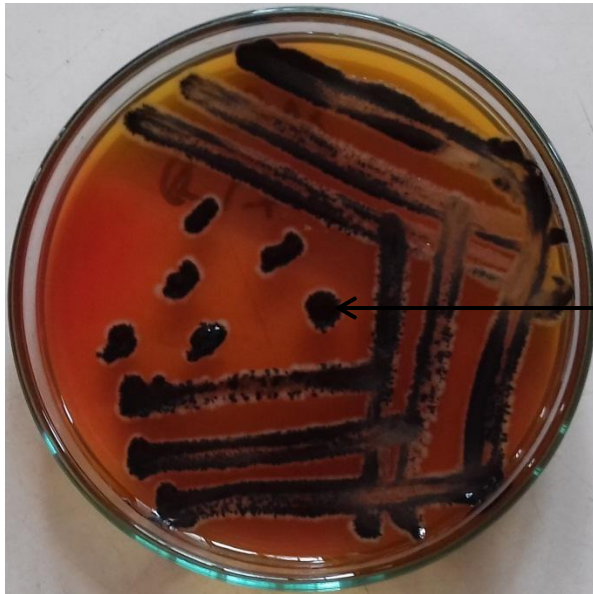


Figure 1. Growth of *Salmonella* spp. on MacConkey agar medium



Figure 2. Growth of *Salmonella* spp. on Salmonella-Shigella agar medium



Small colonies with black centers

Figure 3. Growth of *Salmonella* spp. on Xylose Lysine Deoxycholate agar medium



Pinkish-white colonies surrounded by red zone

Figure 4. Growth of *Salmonella* spp. on Brilliant Green agar medium



Figure 5. Microscopic examination of isolated *Salmonella* spp. in Gram's staining

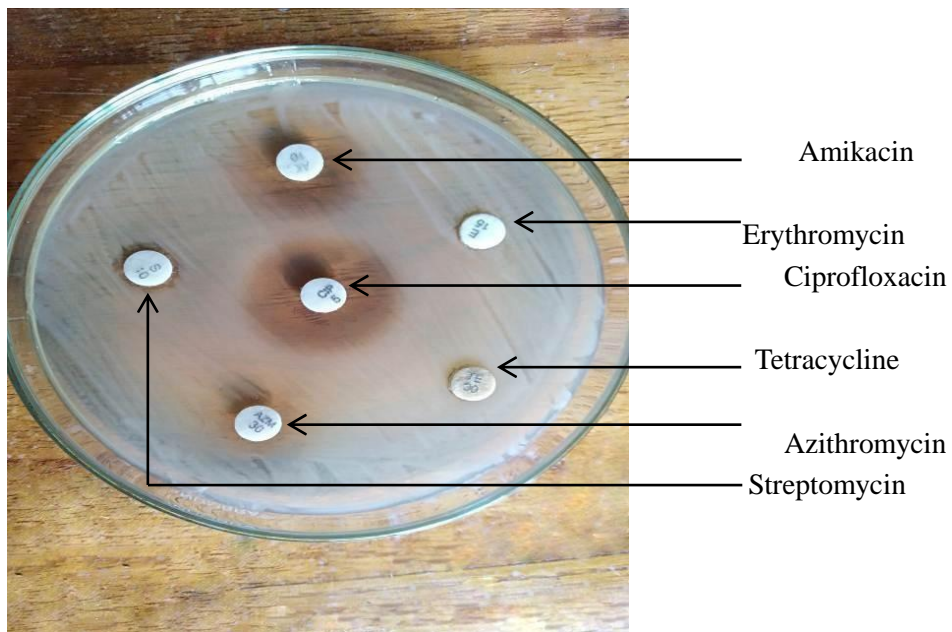


Figure 6. Antibiotic sensitivity test for *Salmonella* spp. on Mueller-Hinton agar medium



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