

Original Article

Molecular detection and antibiogram of *Salmonella spp.* from apparently healthy Japanese quails of three different quail farms in Mymensingh

Shamina Jahan¹, Md Asief Hossain Zihadi¹, K. H. M. Nazmul Hussain Nazir¹, Md. Shafiqul Islam¹, Md. Bahanur Rahman¹ and Marzia Rahman¹#

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AFFILIATIONS

¹Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

CORRESPONDENCE:

Marzia Rahman,
Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
E-mail: marzia_micro@yahoo.com

ABSTRACT

Objective: The present study was carried out for the isolation, identification and antibiogram study of *Salmonella spp.* from apparently healthy Japanese quails (*Coturnix japonica*) at three different quail farms in Mymensingh, Bangladesh.

Materials and methods: A total of 75 cloacal swab samples were randomly collected from apparently healthy Japanese quails from three different farms at Mymensingh, Bangladesh. The samples were subjected to a series of cultural and biochemical examination for the isolation of *Salmonella* followed by molecular detection by polymerase chain reaction (PCR). Motility of the *Salmonella* was performed by motility test and amplification of *speF* gene. The antibiogram profile of the isolates was also evaluated against commonly used antimicrobials by disc diffusion method.

Results: The overall prevalence of *Salmonella spp.* in quails was found to be 13.33%(n=10/75). Out of the 10 isolates, seven were found to be motile. Farmwise, the prevalence of *Salmonella spp.* were 10%(n=3/30), 24%(n=6/25) and 5%(n=1/20) at the quail farms of Bangladesh Agricultural University (BAU), Shikarikanda and Akua, respectively. The antibiogram study revealed that all the isolates were resistant to both Erythromycin and Tetracycline. On the other hand, 100% isolates were sensitive to both Ciprofloxacin and Imipenem. Ninety percent isolates of *Salmonella* were resistant to Colistin sulphate. Neomycin was found to be sensitive to 80% *Salmonella* isolates. All the *Salmonella* isolates were found to be multidrug resistant (MDR).

Conclusion: The presence of MDR *Salmonella spp.* in quails signifies public health importance of the organisms, which may be associated with food-borne illness.

KEYWORDS

Antibiogram; Japanese quail; Multidrug resistant; Prevalence; *speF* gene

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INTRODUCTION

Poultry industry, an excellent agri-business, was started during 1980 in Bangladesh. Although tremendous development of this sector has been occurred in Bangladesh; however, the sector could meet about 68% and 64% of the country's total demand of poultry meat and eggs, respectively. In Bangladesh, poultry meat and eggs provide about 38% of total demand of animal protein (FAO, 1999). To meet up the demand of poultry meat, egg and their products, the Government and the private sector in Bangladesh are working together (Hamid et al 2017).

Quail (*Coturnix japonica*) is a new addition in the poultry industry in Bangladesh, and number of quail farm is increasing day by day due to its easy management, faster growth rate, early sexual maturity, high palatable meat, high nutritional value of meat and egg, high rate of egg production and requirement of less floor space for quail. Quails are more resistant to infectious diseases as compared to chickens although few infectious diseases are encountered in quails. The advancement of quail production is being hampered by some managerial factors, fatal infectious, noninfectious 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). Each year, salmonellosis may cause gastroenteritis and deaths in both developed and developing countries (Kennedy et al., 2004; Majowicz et al., 2010; Rothrock et al., 2015).

Consumption of foods of animal origin such as eggs, egg products, meat, meat products, milk and milk products are the major sources of salmonellosis for human (Behraves et al., 2014; Crump and Heyderman, 2014; McEntire et al., 2014). Avian salmonellosis can be divided into two types; these are pullorum disease caused by *Salmonella pullorum*, and fowl typhoid which is caused by *S. gallinarum* (Barrow and Freitas Neto, 2011). In addition to these two kinds of salmonellosis, birds are also infected by *Salmonella* paratyphoid serovars and other species of *Salmonella*. This may act as potential source of salmonellosis in human (Schoeni et al., 1995; Gast et al., 2013; Behraves et al., 2014).

Antibiotics are essential drugs used to treat infectious diseases of human and animal. In animals, the antibiotics

are used by veterinary surgeons for therapeutic and prophylactic treatment. In some cases, antibiotics are used by farmers and dealers without following any prescription from a registered veterinarian in Bangladesh. In most cases, dose and duration of antibiotic treatment are not followed. As a result, antibiotic resistant bacteria are emerging, which may transmit to humans through food items originated from poultry. Most of the people acquire food-borne infections by multidrug resistant (MDR) *Salmonella* by consumption of contaminated foods of animal origin (Angulo et al., 2000). Many researchers studied the prevalence of *Salmonella*, isolated and detected the *Salmonella* from chickens in Bangladesh (Barua et al., 2012; Naurin et al., 2012; Sultana et al., 2014; Mahmud et al., 2015; Parvej et al., 2016; Akter et al., 2017). Very few researches have been performed on quail *Salmonella* (Sander et al., 2001; Bacci et al., 2012). There is paucity of literature that describes the isolation and molecular detection of *Salmonella* from Japanese quail in Bangladesh.

Though quails are naturally disease resistant but they may act as reservoir of pathogens for other birds located in the same or surrounding farm. Considering this point of view, it is necessary to investigate the association of *Salmonella* with quail. This study was aimed at molecular detection and antibiogram study of *Salmonella* from Japanese quails for the first time in Bangladesh.

MATERIALS AND METHODS

Ethical statement: Cloacal swab samples from the quails were collected as per the international standard considering animal welfare giving minimum pain or discomfort to the birds.

Collection and pre-enrichment of samples: Swab samples were directly collected from cloaca of apparently healthy Japanese quails of three quail farms including Bangladesh Agricultural University (BAU) poultry farm (n=30), Akua (n=20) and Shikarikanda (n=25) in Mymensingh. Each swab was placed aseptically into test-tube containing nutrient broth (NB) for pre-enrichment. The samples were transported to the Bacteriology Laboratory at the Department of Microbiology and Hygiene, BAU.

Isolation and identification of *Salmonella*: The NB containing pre-inoculated sample was incubated at 37°C for enrichment. Two hundred microliter of the enriched sample was spread onto *Salmonella*-Shigella (SS) and Xylose Lysine Deoxycholate (XLD) Agar (Hi-media, India), and was incubation aerobically at 37°C overnight.

Table-1: Oligonucleotide primers used in the study

Name of primers	Primer sequence (5'-3')	Size of amplicon	References
Sal 16SrRNA-F	TGTTGTGGTTAACCGCA	574-bp	Lin and Tsen (1996)
Sal 16SrRNA-R	CACAAATCCATCTCTGGA		
<i>SpeF</i> -F	TTAGCCGTCATTGCCCGGATT	2000-bp	Ribeiro et al. (2009)
<i>SpeF</i> -R	ACGAGGTTTAATGACGTAGC		

Table 2: Prevalence of *Salmonella* spp in three different quail farms in Mymensingh

Name of Farm	No. of Sample examined	No. of Positive sample	Prevalence of <i>Salmonella</i> spp (%)
BAU poultry Farm	30	3	10
Akua	20	1	5
Shikarikanda	25	6	24
Total	75	10	13.33

Typical colonies showing characteristics pink to red color with black center were suspected as *Salmonella*, which were subjected for sub-culturing onto XLD agar to obtain pure cultures. The isolates were confirmed as *Salmonella* by cultural and morphological characterization by Gram stain, biochemical characterization, followed molecular detection by PCR using *Salmonella* specific primers. The biochemical tests include motility indole urease (MIU), sugar fermentation, Methy Red (MR), Voges-Proskauer (VP) and indole production tests were carried out for identification of *Salmonella* spp. ([Kabir et al., 2017](#)).

Amplification of 16S rRNA and *speF* gene: For the amplification of 16S rRNA and *speF* genes of *Salmonella*, template DNA was extracted by boiling method ([Queipo-Ortunet et al., 2008](#)). Briefly, a single colony of each isolate was picked up and dissolved in 100 µL of distilled water, which was boiled for 10 min. After boiling, the samples were kept into ice for heat shock for 10 min. The cell lysate were spined down for 5 min at 10,000 rpm for pelleting the cellular debris. The supernatant was collected and used as template for amplification of target genes by PCR. The primers used in this study are presented in **Table 1**.

To amplify the 16SrRNA and *speF* genes in the isolated *Salmonella*, the reaction mixture was prepared in a total volume of 25 µL containing 12.5 µL PCR master mix (Promega, USA), 10 pmol of each primer and 5 µL of template DNA. The standard conditions for amplification of 16SrRNA gene were- 35 cycles with initial incubation at 94°C for 5 min, denaturation at 94°C for 60 Sec, annealing at 50°C for 30 Sec, elongation at 72°C for 30 Sec and final extension for 5 min at 72°C. For *speF* gene amplification, the conditions were- 25 cycles with initial incubation at 94°C for 5 min, denaturation at 92°C for 30 Sec, annealing at 50°C for 60 Sec and elongation at 72°C for 5 min. The PCR products were analyzed by

electrophoresis using 1.5% agarose gel followed by staining with ethidium bromide and visualized by UV trans-illuminator (Biometra, Jena, Germany).

Antibiogram study: The antibiotic sensitivity patterns of all *Salmonella* isolates were performed by standard disc-diffusion method according to the method described by [Saifullah et al. \(2016\)](#). This test was carried out to assess antibiogram patterns of the isolates against Erythromycin (15 ug), Tetracycline (30 ug), Ciprofloxacin (5 ug), Imipenem (10 ug), Amoxicillin (30 ug), Colistin sulphate (10 ug) and Neomycin (30 ug) (HiMedia, India). The antibiogram patterns were interpreted as per the guidelines of [CLSI \(2008\)](#).

RESULTS

A total of 75 cloacal swabs of quail were analyzed for the presence of *Salmonella*. The present study showed the overall prevalence of *Salmonella* spp. as 13.33%(n=10/75). The highest and lowest incidence of *Salmonella* were 24% and 5% found in the quail farms in Shikarkanda and Akua, respectively. (**Table 2**) *Salmonella* produced small, round, smooth and black centered colonies onto SS agar and pink color colony with black center on XLD agar. A total of 10 isolates were confirmed as *Salmonella* spp. by amplification of 16SrRNA gene (**Figure 1**) using genus specific primers. Out of 10 isolates, three were confirmed as non-motile after performing motility test and PCR of *speF* gene (**Figure 2**).

Antibiogram study of all *Salmonella* isolates revealed that all the isolates were MDR. All the isolates were found resistant to Tetracycline and Erythromycin and 90% isolates were resistant to Colistin sulphate. All the isolates were found 100% sensitive to Ciprofloxacin and Imipenem followed by Neomycin (80%) and Amoxicillin (60%). The resistance or sensitivity patterns of the isolated *Salmonella* isolates against antibiotics are

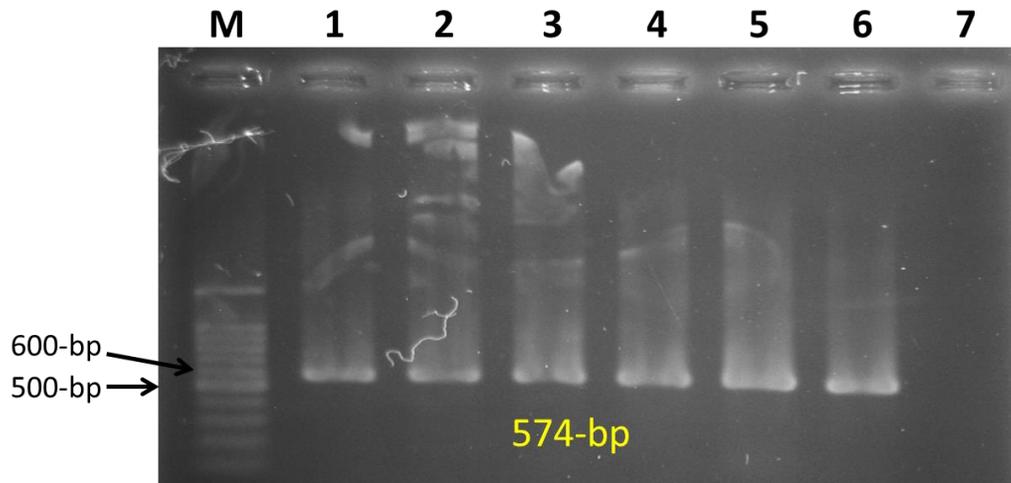


Figure 1. Amplification of *16SrRNA* gene of *Salmonella* spp. (574-bp) (Lane M 100 bp ladder, lane 1-6 *Salmonella* spp. isolates with positive amplicon, and lane 7 is negative control).

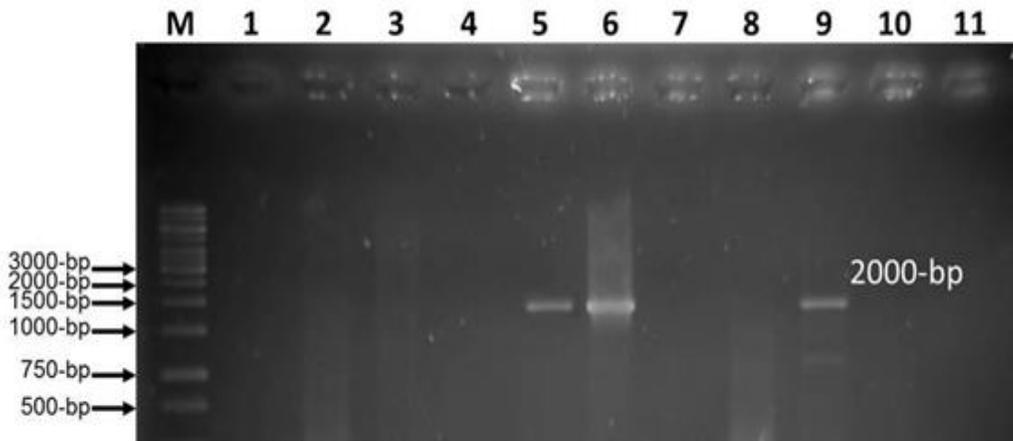


Figure 2. Amplification of *speF* gene of non-motile *Salmonella* (2000-bp). (Lane M 1kb ladder, lane 5, 6, and 9 positive for *speF* gene, 11 negative control).

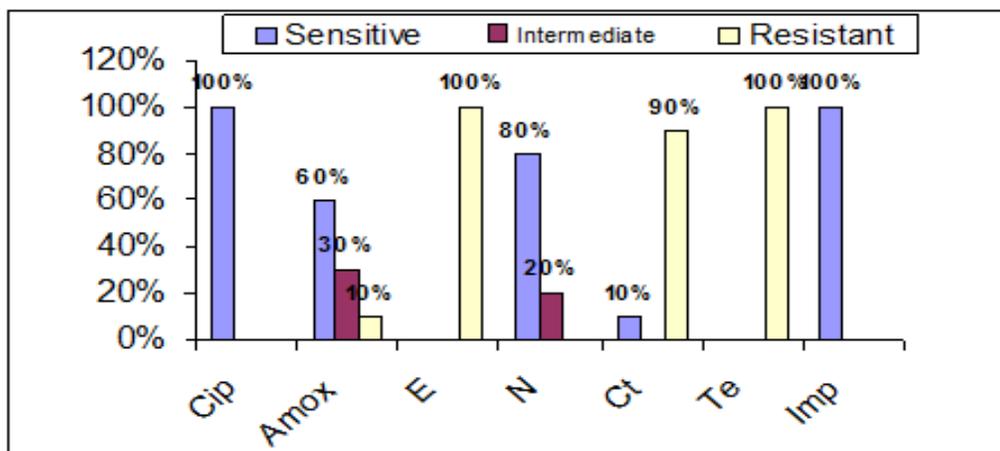


Figure 3. Antibiogram profile of *Salmonella* spp. isolates of quail against different antibiotics. *Cip*=Ciprofloxacin, *Amox*=Amoxicillin, *E*=Erythromycin, *Ct*=Colistine sulphate, *Te*=Tetracycline, *Imp*=Imipenem.

illustrated in **Figure 3**.

DISCUSSION

Salmonellosis is one of the most serious problems for both human and animal in developing countries including Bangladesh, because the climatic conditions favor the environmental spread of these organisms leading to increased incidence of salmonellosis. For that reason, it is important to know the source of *Salmonella* that may cause infection in both human and animal. Several serotypes of *Salmonella spp.* are reported to be pathogenic for both human and animal such as *Salmonella typhimurium*, *Salmonella enteritidis* (Kennedy et al., 2004; Hendriksen et al., 2011). Since the isolation and accurate identification of *Salmonella spp.* are crucial for their characterization, in this study, *Salmonella spp.* from the quails were isolated and identified on the basis of cultural and biochemical properties followed by molecular detection using genus specific primers. Non-motile biovars, viz., *S. gallinarum* and *S. pullorum* were identified by amplification of *speF* gene that is specific for non-motile *Salmonella* and motility test. Many authors isolated and identified pathogenic species of *Salmonella* from quails (Sander et al., 2001; Bacci et al., 2012) in different countries.

There are many reports available on the isolation and molecular detection of *Salmonella* from chicken and other animals in Bangladesh (Barua et al., 2012; Parvej et al., 2016; Saifullah et al., 2016). However, before this report, no reports were available on isolation and molecular identification of *Salmonella* from Japanese quail.

In this study, the prevalence of *Salmonella spp.* was found to be 13.33% (n=10/75), which is similar to the report of Palanisamy and Bamaiyi (2015). Several authors could not be able to isolate *Salmonella* from quail (McCrea et al., 2006; Dipineto et al., 2014) whereas, some others could isolate *Salmonella* from quail (Sander et al., 2001; Bacci et al., 2012; Udhayavel et al., 2016).

Variation in prevalence of *Salmonella* among the three farms was observed (Table 2). This variation might be due to the variation in managerial practices. Unhygienic management may act as the cause of high prevalence of *Salmonella*. The presence of motile and non-motile *Salmonella* were identified by PCR of *speF* gene which harbors in *S. gallinarum* and *S. pulorum*. Out of 10 isolates, only three were found as non-motile. This finding is in agreement with the report of Parvej et al. (2016) who found 11 motile *Salmonella* among 150 samples.

In our study, the isolated *Salmonella* showed resistant to Tetracycline, Erythromycin and Colistine sulphate, indicating that the isolates were MDR. The findings of this study were similar to the report of Hyeon et al. (2011). The isolates showed 90% resistant to Colistine sulphate, but Parvej et al. (2016) found 50% isolates were resistant to Colistine sulphate and 80% were sensitive to Neomycin. This indicated that Colistin sulphate is becoming resistant due to indiscriminate and unwise use. All the isolates of three farms showed 100% sensitivity towards Ciprofloxacin, as reported by Ramya et al. (2013), who found 100% susceptibility of *Salmonella spp.* to Ciprofloxacin followed by Amoxicillin (82%). 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).

CONCLUSION

Salmonella spp. have been successfully isolated and identified from cloacal swab of apparently healthy Japanese quail for the first time Bangladesh. The isolates are confirmed as *Salmonella spp.* by PCR. The overall prevalence is 13.33%(n=10/75). Antibioqram study reveals the presence of MDR *Salmonella spp.* in healthy quail which may be transferred to the humans and animals.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

SJ, MR, and MSI designed the project. SJ and MAHZ performed the actual experiments. SJ drafted the first version of the manuscript. MR, MSI finalized the manuscript. MBR, MR and KHMNHN critically reviewed the article and finally approved for publication.

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