

## Prevalence and antibiogram profile of *Mycobacterium* spp. in poultry and its environments

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### ABSTRACT

In this study, an attempt was undertaken to know the prevalence and antibiogram profile of *Mycobacterium* spp. in poultry and its immediate environments. A total of 130 samples comprising of droppings (n=80), egg washing (n=18), drinking water (n=14), hand washing from farm workers (n=6) and litter (n=12) were collected from six poultry farms located in and around Bangladesh Agricultural University (BAU). Samples were inoculated onto 7H10 Middlebrook agar and incubated aerobically at 37°C for 7-14 days. Identification of *Mycobacterium* spp. was performed by colonial morphology, acid fast staining, and biochemical tests. Molecular identification of *Mycobacterium* spp. at genus level was performed by polymerase chain reaction (PCR) assay targeting 65-kDa heat shock protein gene. Antibiogram profile of *Mycobacterium* spp. was performed against five antibiotics namely Rifampin, Azithromycin, Ciprofloxacin, Streptomycin and Doxycycline by disc diffusion method. Three *Mycobacterium* spp. were isolated from dropping samples of poultry. The overall prevalence of *Mycobacterium* spp. was 2.3% (n=3/130). All the isolates were resistant to Rifampin and sensitive to Azithromycin and Ciprofloxacin. Data of this study indicated that multidrug resistant *Mycobacterium* spp. are prevalent in the poultry farms of the study area which underscore the need of implementation of good biosecurity to poultry husbandry practice to ensure poultry and human health.

### Keywords

Antibiogram profile, *Mycobacterium* spp., Poultry farm, Polymerase chain reaction

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### INTRODUCTION

Avian tuberculosis (TB) is a bacterial zoonotic disease (Radostits et al., 2000) globally which has been reported in pet, (Chadha, 2009) free-living (Dvorska, 2007) and captive wild birds (Hejlíček and Tremi, 1995) and poultry (Martin and Schimmel, 2000). Avian tuberculosis (ATB) is most commonly caused by *Mycobacterium avium* subsp. *avium*, and less frequently by *M. genavense* (Pavlik et al., 2000; Tell et al., 2011). Other *Mycobacteria* such as: *M. intracellulare*, *M. scrofulaceum*, *M. fortuitum*, *M. tuberculosis*, and *M. bovis* rarely cause avian TB (Fulton et al., 2003). *Mycobacteria* are aerobic, acid fast, straight or curved rod, mesophilic bacteria (Madigan et al., 2003).

Avian TB caused by *M. avium* reported in wide range of avian species including waterfowl, galliformes, columbiformes, passerines, psittacines, raptors, and ratites (Fulton et al., 2003; Dvorska et al., 2007). *Mycobacterium* is able to survive in the environment for long time. Infected birds, contaminated soil and water are the major sources of transmission of infection in birds, animal and humans (Fulton et al., 2003). The incidence of tuberculosis is higher in densely populated areas where hygienic and sanitary conditions are unsatisfactory. Free ranging birds and old breeders very often spread tuberculosis (Dhama et al., 2007).

Infected birds are the main source of infection since they are known to excrete bacteria via feces into the environment. Susceptible birds get infected through ingestion and inhalation of aerosolized bacteria. Long time surviving ability of the bacteria in soil and litter favor rapid transmission of tuberculosis to the birds (Tell et al., 2001). Contaminated equipments, pens pasture, attendant's clothing, hands and feet play an important role in the transmission of disease (Tell et al.,

2001). Avian TB is uncommon in commercial broiler farms and in layers the infection is very common (Fulton et al., 2003).

An increase in antibiotic resistance of the genus *Mycobacterium* particularly to antibiotics has been reported (Dhama et al., 2011). The infected bird must be treated for a long period using combination drug treatment. Eradication is difficult due to carrier birds and frequent shedding of bacteria from the feces (Fulton et al., 2003).

In Bangladesh, no study has been conducted to know the prevalence and antibiogram profile of *Mycobacterium* spp. from poultry and its environments. The objectives of this study were (i) isolation and identification of *Mycobacterium* spp. from poultry droppings, egg washing, drinking water, hand washing of poultry farm workers, and litter by routine bacteriological methods and polymerase chain reaction (PCR) assay, and (ii) determination of antibiogram profile of *Mycobacterium* spp. against five antibiotics such as: Rifampin, Azithromycin, Ciprofloxacin, Streptomycin and Doxycycline.

## MATERIALS AND METHODS

**Sample collection and transportation:** Dropping of poultry was collected by a sterile cotton swab. The swab containing fecal sample was placed in a test tube containing nutrient broth. Surface of egg was washed with 50 mL PBS kept in sterile polyethene bag. 10mL of egg washing was placed in a screw capped test tube. Drinking water for poultry was collected from water trough by a 20 mL sterile disposable syringe. 10mL of water sample was placed in a screw capped test tube. Hands washing of poultry farm workers were washed by sterile PBS. 10 mL of hand washing sample was placed in a screw capped test tube. 50 gm of litter sample was collected from the area where poultry are kept. Then litter was placed in a test tube containing nutrient broth. All of the above samples were kept in an ice box and transported to the Department of Microbiology and Hygiene, BAU, Mymensingh for bacteriological analysis.

**Processing and enrichment of samples:** Samples were processed for bacteriological analysis immediately after arrival to the bacteriological lab. At first samples were vortexed separately and then it was enriched in nutrient broth and incubated at 37°C overnight.

**Isolation of bacteria:** Samples were enriched separately in nutrient broth and incubated overnight at 37°C. The

overnight enrichment culture was streaked onto 7H10 Middlebrook agar and incubated at 37°C for 7 days. Single colony grown onto the 7H10 Middlebrook agar was further sub cultured onto 7H10 Middlebrook agar until pure culture was found.

**Identification of bacteria:** Bacteria were identified by cultural characteristics and colony morphology on the 7H10 Middlebrook agar. Acid-fast and (Shoeb, 2005), endospore staining techniques (Lamont et al., 2012), motility test using hanging drop method (Anderson et al., 2005), biochemical tests (catalase test and oxidase test) were performed to identify bacteria at genus level.

**Molecular detection of bacteria by PCR:** A genus specific PCR assay was performed to identify *Mycobacterium* spp. by amplifying 439-bp fragment of the gene encoding for 65-kDa heat shock protein, as described by Telenti et al. (1993).

**Antibiogram study:** Three isolates were tested for antimicrobial drug susceptibility against 5 antibiotics such as: Rifampin (5 µg/disc), Azithromycin (30 µg/disc), Ciprofloxacin (5 µg/disc), Streptomycin (10 µg/disc) and Doxycycline (30 µg/disc) by disc diffusion method or Kirby-Bauer method (Bauer et al., 1966). Briefly, 4 to 5 single bacterial colonies grown on 7H10 Middlebrook agar were taken into 5 mL of nutrient broth and incubated for 24 h at 37°C. One mL test culture was homogeneously poured onto Mueller-Hinton agar (Himedia, India). Antimicrobial discs (Oxoid Ltd., Hampshire, England) were placed onto Mueller-Hinton agar with the help of sterile forceps. The plates were incubated for 24 h at 37°C. The diameter of the zone of complete inhibition (including diameter of the discs) after incubation was measured in millimeters by a meter ruler. Antibiogram profiles of the isolates were determined according to standard guidelines described in the manual of the Clinical and Laboratory Standard Institute (CLSI, 2012).

**Statistical analysis:** Prevalence of *Mycobacterium* in various poultry and its environmental samples were analyzed by Sqi-square ( $\chi^2$ ) test for statistical significance using SPSS version IBM 20. A *P* value of  $\leq 0.05$  was considered as statistically significant.

## RESULTS AND DISCUSSION

*M. avium* is known to infect poultry. It can also cause infection in immunocompromised humans. In this study, an effort was undertaken to know the prevalence of *Mycobacterium* spp. in poultry and its

immediate environments. The study also assessed public health impact of *Mycobacterium* spp. on poultry farm workers. *M. avium* infection has a little impact on the poultry production and human disease (Martin and Schimmel, 2000).

In this study, droppings of poultry, egg washing, drinking water, hand washing of poultry farm worker and litter of poultry farm were screened for detection of *Mycobacterium* using standard bacteriological method (Telenti et al., 1993). The 7H10 Middlebrook agar medium was used for isolation of *Mycobacteria* from all samples since it is a selective medium for *Mycobacteria* (Dhama et al., 2007). On 7H10 Middlebrook agar culture positive samples produced small, smooth, sticky, round and off-white color colonies which were characteristics of the genus *Mycobacterium* (Griffith et al., 2007; Dhama et al., 2007, Tell et al., 2011).

*Mycobacterium* is a rod shaped, non spore forming, non motile, acid fast bacterium (Pfyffer, 2007; Brown-Elliott and Wallace, 2007; Vincet and Gutierrez, 2007). In this study, colony grown on 7H10 Middlebrook agar were examined by acid fast staining, spore staining and hanging drop slide techniques. The results indicated that isolates recovered from poultry farm were *Mycobacteria* since these are acid fast, non-spore forming and non-motile. Identification of the bacteria at the molecular level was also confirmed by a genus specific PCR assay targeting *hsp* 65-kDa gene (Telenti et al., 1993). Although routine bacteriological study confirmed three *Mycobacterium* isolates in the dropping sample but PCR assay only amplified 439-bp amplicon of 65-kDa genes from two culture positive DNA samples (Figure 1). One isolate did not yield 439-bp amplicon in PCR assay which might be resulted from low yield of DNA from *Mycobacterium* (Awua et al., 2010). In this study, positive control DNA of *Mycobacterium* was not available to use in the PCR assay. The identity of *Mycobacterium* at species level was not confirmed which need further study.

In this study, *Mycobacterium* isolates were only isolated from the dropping samples of poultry. The prevalence of *Mycobacterium* spp. in the dropping samples was 3.75% (n=3/80). Out of three *Mycobacterium* isolates; one was isolated from dropping of broiler and rest two isolates were recovered from two farms such as: , Ratan Poultry Farm and Ashik Poultry Farm. The prevalence of *Mycobacterium* spp. in the dropping samples was 25% (n=1/4) at BAU Poultry Farm, 5% (n=5/20) in Ratan Poultry Farm and 8.33% (n=1/12) in Ashik

Poultry Farm (Figure 2). Inadequate hygienic and biosecurity measures was observed at BAU poultry farm as compared to other poultry farms in the study areas which might be responsible for higher prevalence of *Mycobacterium* at BAU poultry farm. *Mycobacterium* infection is more common in older birds as compared to younger birds (Soler et al., 2009). In this study, prevalence of *Mycobacterium* spp. in broiler (age less than 35 days) was 1.61% (n=1/62) and in layer (age 20 weeks), it was 2.94% (n=2/68) (Figure 3).

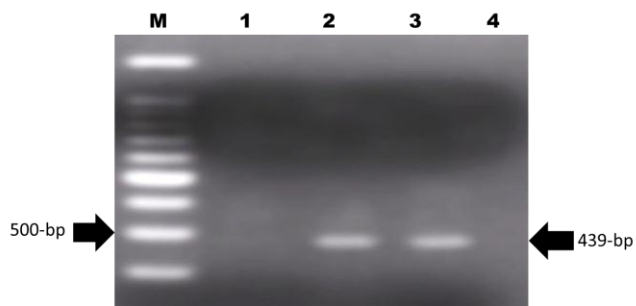


Figure 1. Amplification of *hsp* 65 kDa gene of *Mycobacteria* by PCR. Lane M: 250bp size DNA ladder. Lane 1, 2 and 3: DNA of three culture positive *Mycobacterial* isolates. lane 1, DNA isolate of broiler did not amplify. Lane 4: Negative control without DNA.

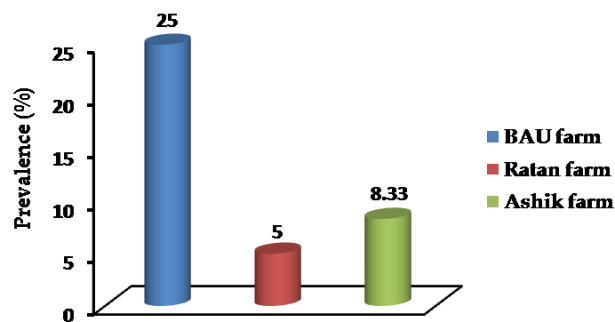


Figure 2. Prevalence of *Mycobacterium* spp. in dropping samples in BAU Poultry Farm, Ratan Poultry Farm and Ashik Poultry Farm.

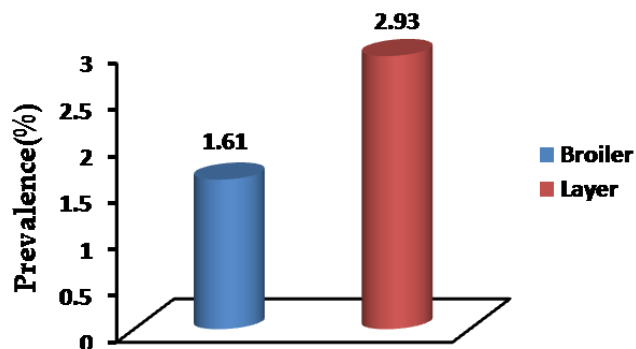


Figure 3. Prevalence of *Mycobacterium* spp. in layer and broiler birds.

**Table 1. Prevalence of *Mycobacterium* spp. in samples of poultry and its environments**

Serial No.	Name of samples	No. of samples tested	No. of <i>Mycobacteria</i> Positive samples	Prevalence (%)	Overall prevalence (%)	P-value
1	Droppings	80	3	3.75		
2	Egg washing	18	0	0		
3	Drinking water	14	0	0	2.31	0.00
4	Hand washing	06	0	0		
5	Litter	12	0	0		

**Table 2: Summary of antibiogram profile of *Mycobacterium* spp. against five antibiotics**

No. of isolate tested	Antibiogram profile of <i>Mycobacterium</i> spp.														
	RIF			AZM			CIP			STM			DOX		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
3	3	0	0	0	0	3	0	0	3	1	0	2	0	2	1

Legend: S= Sensitive, I= Intermediate, &R= Resistant; RIF= Rifampin, AZM= Azithromycin, CIP =Ciprofloxacin, STM= Streptomycin and DOX= Doxycycline.

*Mycobacterium* causes significant infection in layer farms where high age group birds are maintained (Dhama et al., 2011). A study conducted in Netherland recorded 16% prevalence of *Mycobacterium* in the droppings of broiler chickens (Smitet al., 1987). Another study performed in Switzerland recorded 5% prevalence of *Mycobacterium* in the droppings of layer chickens (Alexander et al., 1968). Infected birds known to excrete bacteria in feces and may spread from bird to bird and from bird to animals and humans (Martin et al., 2000). Feces containing *Mycobacteria* may contaminate litter, drinking water and egg surface (Alexander et al., 1968). In this study, *Mycobacteria* were not recovered from none of the above samples indicated that it was not wide spread bacteria in this poultry farms of this study. *Mycobacteria* were not isolated from hand washing of the poultry farm workers indicated that these bacteria may not pose public health threat.

The overall prevalence of *Mycobacteria* in this study was 2.31% (n=3/130) among all poultry farms and these bacteria were only found in the droppings of poultry indicated a few number of birds might have shedded this bacterium (Table 1).

Emergence of multi-drug resistance *Mycobacterium* is a serious public health issue throughout the world including Bangladesh (Chadha, 2009; Flora et al., 2013). In this study, antibiogram profile of *Mycobacterium* isolates of poultry were carried out against five antibiotics such as: Rifampin, Azithromycin, Ciprofloxacin, Streptomycin and Doxycycline. These antibiotics are used to treat intracellular bacteria like *Mycobacterium* infection in human (Vincet et al., 2007).

Rifampin and Streptomycin are considered as two important anti-tuberculosis drugs (Shi et al., 2007). All isolates of the present study were found resistant to Rifampin. However, only one isolate in this study showed multi-drug resistance profile since it was found resistant to Rifampin and Streptomycin (Table 2 and Figure 4). In another study, Rifampin and Streptomycin resistant *Mycobacterium* strains were reported by Affolabi et al. (2009).

Data of this study indicated that multi-drug resistant *Mycobacterium* spp. was prevalent in the poultry farm which underscored the need of implementation of good biosecurity to poultry husbandry practice to protect the poultry and human health.

## CONCLUSION

Results of this study indicated that all the three isolates of *Mycobacterium* spp. were sensitive to Azithromycin and Ciprofloxacin but were resistant to Rifampin. Current study indicated that multi-drug resistant *Mycobacterium* spp. is prevalent in poultry farms of the study area which may cause health hazard to immune-compromised individuals.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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