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Original Article

Detection of antibiotic resistant *Avibacterium paragallinarum* from broiler chickens in Bangladesh

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ABSTRACT

Objective: An attempt was undertaken for the detection and characterization of *Avibacterium paragallinarum* from clinically sick broiler chickens during field outbreaks.

Materials and methods: Nasal and ocular discharges (n=6), tracheal swab (n=6), tracheal washing (n=4) and infraorbital sinus exudates (n=4) were collected aseptically from broiler chickens (n=10). To isolate *A. paragallinarum*, the clinical samples were cultured onto blood agar and chocolate agar enriched with Nicotinamide Adenine Dinucleotide (NAD) and feeder organism (*Staphylococcus aureus).* Identification of *A. paragallinarum* was performed by Gram staining reaction, sugar fermentation profiles using five basic sugars (Dextrose, Maltose, Sucrose, Lactose and Mannitol) and biochemical tests (Indole, Voges Proskauer and Methyl red tests). Antibiogram of the bacterial isolates of infected chicken was performed against five antibiotics namely Ciprofloxacin, Azithromycin, Gentamicin, Ampicillin and Cefalexin using disk diffusion method.

Results: Results of colonial morphology, Gram staining reaction, sugar fermentation and biochemical tests confirmed one isolate as *A. paragallinarum*. The overall prevalence of IC in broiler chicken was 10% (1 of 10). This isolate was found to be sensitive to Ciprofloxacin, Azithroycin and Gentamicin and resistant to Ampicillin and Cefalexin.

Conclusion: This is the first report of detection of *A. paragallinarum* from broiler chicken in Bangladesh.

KEYWORDS

Avibacterium paragallinarum, Broiler, Infectious coryza, Multidrug resistant

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INTRODUCTION

Infectious coryza (IC) is a contagious bacterial disease of poultry caused by *Avibacterium paragallinarum* It is a common bacterial disease in the commercial poultry (Gayatri et al., 2010). It mainly affect the upper respiratory tract of chickens The meat of the affected chicken is condemned if it is infected with *A. paragallinarum* (Blackall et al., 2005). The disease causes significant economic losses in broiler farms (Droual et al., 1990).

Chicken infected with IC manifested the clinical signs of nasal discharge, conjunctivitis and swelling of the sinuses, face and wattles. *A. paragallinarum* causes catarrhal inflammation of the upper respiratory tract mainly in the sinuses and nasal passage (Blackall, 1999). The epidemmiology of IC is very complex. The outbreak of IC is common in farms that keep birds of various ages together. Over crowding, cold weather and co-infection with pathogenic microorganisms (*Pasteurella multocida*, *Pesudomonas aeruginosa* and chronic respiratory disease) determine the incidence of the IC in the poultry farm (Blackall, 1999; Reid and Blackall, 1984; Giurov, 1984). The mortality of chicken due to IC varies from 1 to 15% and it can be increased if birds are co-infected with other microorganisms (Sarhiland et al., 2003).

The IC has been reported in layer chicken in Bangladesh (Talha et al., 2001, Akthar et al., 2001; Akter et al., 2013; Akter et al., 2014). To the best of our knowledge there is no report on the detection of *A. paragallinarum* in broiler chicken in Bangladesh. The objectives of this study were: (i) to isolate and identify *A. paragallinarum* from broiler chicken using cultural, staining and biochemical methods and (ii) to determine antibiogram profiles of *A. paragallinarum*.

MATERIALS AND METHODS

Birds and study areas: Broiler chickens (n=10) manifested the characteristic clinical signs of IC were collected from four poultry farms located at Puthia and Baghmara Upazilla of Rajshahi district, Natore sadar and Mymensingh sadar Upazilla during the period from November 2013 to February 2014. All birds belonged to 4 to 5 weeks of age.

Transportation of birds: Live birds (n= 6) were placed in cages and dead birds (n=4) were packed in ice boxes and transported to the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh.

Collection of samples: Nasal and ocular (n=6) and tracheal (n=6) swabs were aseptically collected from live birds (n=6) using sterile cotton swabs. Tracheal washing (n=4), tracheal swab (n=4) and swab of infraorbital sinus (n=4) were aseptically collected from dead birds (n=4).

Enrichment of samples: Clinical specimens collected from live and dead birds were enriched in Nicotinamide Adenine Dinucleotide (NAD) added glycerol-phosphate buffer saline by incubation at 37^{0}C for 24 h (Byarugaba et al., 2013).

Isolation and identification of bacteria: A loopful of enrichment culture was streaked onto blood agar and chocolate agar supplemented with NAD, feeder colony (*Staphylococcus aureus*) and incubated anaerobically in a candle jar at 37^{0}C for 24 h. Well isolated single colony was further sub cultured until pure culture was obtained. Identification of bacteria was performed by colony morphology, Gram's staining reaction, motility test, sugar fermentation and biochemical tests (MR-VP, Indole and Catalase).

Antibiogram profile: Gentamicin (10 µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Ampicillin (10 µg) and Cefalexin (30 µg) were used to know the antibiogram profileof *A. paragallinarum*. The antibiotics susceptibility testing was performed according to the guideline of CLSI (2007).

RESULTS AND DISCUSSION

Poultry industry throughout the world suffers a huge economic loss due to IC. *A. paragallinarum* is the etiological agent of this disease. It causes infection in the upper respiratory tract of chickens (Blackall et al., 2005). To prevent the economic losses due to IC, it is necessary to detect and characterize the causal agent of the disease. The present research work was done to identify *A. paragallinarum* in broiler chickens manifested clinical signs of IC during natural outbreaks.

In this study, birds manifested the clinical signs of ocular and nasal discharges, conjunctivitis, infraorbital swelling, facial swelling, and open mouth breathing. Similar clinical sign of birds affected by IC were also reported by Sakamoto et al. (2013). Out of 10 clinical samples, one culture positive sample was confirmed as *A. paragallinarum*. The overall prevalence of *A. paragallinarum* in this study was 10%. A study conducted in India reported 0.6% prevalence of *A. paragallinarum* in broilers (Durairajan et al., 2013). In this study samples obtained from nine birds were culture negative although these birds manifested the clinical signs of IC. Other than *A. paragallinarum* several microbial agents such as *P. multocida*,

Table 1. Antimicrobial sensitivity assay of *A. paragallinarum*

Antibiotics	Inhibition zone (mm)	Interpretation
Gentamicin	28	S
Azithromycin	20.2	S
Ciprofloxacin	30	S
Ampicillin	-	R
Cefalexin	-	R

S= Sensitive; R= Resistant; (-) = No zone of inhibition

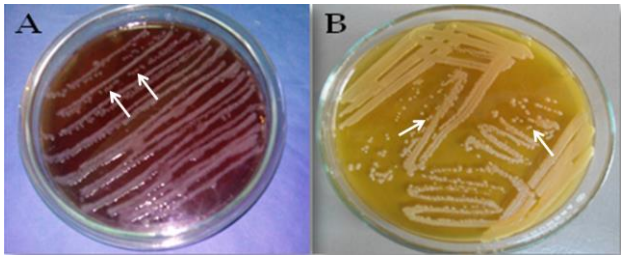

Figure 1. (A) Frequent tiny drop colonies (arrows) of *A. paragallinarum* are seen adjacent to the feeder organism on NAD supplemented blood agar. (B) Dewdrop colonies (arrows) of *A. paragallinarum* are seen adjacent to the feeder colony as well as throughout colonies on NAD supplemented chocolate agar.

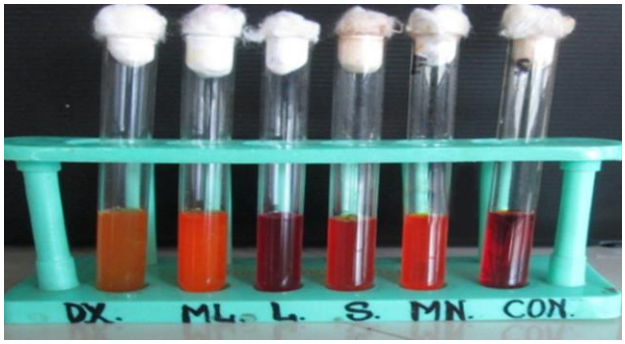

Figure 2. Sugar fermentation tests. *A. paragallinarum* produces acid by fermenting dextrose (DX), maltose (ML), sucrose (S) and mannitol (MN). No fermentation was seen in the cases of lactose (L) and control (CON).

Figure 3. Biochemical tests by *A. paragallinarum*. The bacteria produce orange and yellow colors in Methyl Red (MR) and Voges-Proskauer (VP) test indicating negative results, repectively. No color was seen in indole test and coltrol.

P. aeruginosa, fowl pox and chronic respiratory disease (CRD) are also known to cause upper respiratory infections in poultry (Giurov, 1984). Additionally, administration of antibiotics to the clinically sick chickens on the affected farms might also be responsible for non-isolation of *A. paragallinarum* from the samples of nine the affected chickens.

In the present study, *A. paragallinarum* were recovered from ocular, nasal and tracheal swabs. Similar clinical specimens were also used for isolation of *A. paragallinarum* by Akter et al. (2014) and Blackall (1999). *A. paragallinarum*is a fastidious bacterium. In this study, V factor and NAD were used for the isolation of *A. paragallinarum*. Soriano and Terzolo (2004) also used V factor and NAD for the isolation of *A. paragallinarum* in their research. In blood agar, satellite colonies were seen adjacent to the feeder colony (*S. aureus*) (**Figure 1-A**) and in chocolate agar dewdrop colonies were seen adjacent to the feeder colonies (**Figure 1-B**). Similar cultural characteristics were also reported by several investigators (Akter et al., 2014; Chukiatsiri et al., 2010; Christensen et al., 2009).

The growth of the organism on NAD enriched chocolate agar and no growth of organism on chocolate agar without NAD confirmed the identity of bacteria as *A. paragallinarum* (Chukiatsiri et al., 2010). In Gram staining, *A. paragallinarum* were seen as Gram negative small rod or cocco-bacilli. These staining and morphological characterristics were also reported by Jaswinder et al. (2004) and Sameera et al. (2001).

Only one isolates of *A. paragallinarum* fermented dextrose, sucrose maltose, and mannitol (**Figure 2**). Similar sugar fermentation reaction profile of *A. paragallinarum* were also described by Blackall and Yamamoto (1989), Hinz and Kunjara (1977), Sameera et al. (2001), Jaswinderet al. (2004) and Durairajan et al. (2013). In this study, biochemical tests reaction were negative for MR-VP, Indole and catalase tests (**Figure 3**) which confirmed the isolate as *A. Paragallinarum* (Akter et al., 2013; Sameera et al., 2001; Jaswinder et al., 2004; Thenmozhi and Malmarugan, 2013).

In the present study, *A. paragallinarum* isolates exhibited resistant antibiogram profile against Ampicillin and Cephalexin. The isolate showed sensitive profile to Ciprofloxacin, Azithromycin and Gentamicin. The result of antimicrobial sensitivity assay is presented in **Table 1**. Almost similar antibiogram profiles of *A. paragallinarum* were also recorded by Haunshi et al. (2006), Sameera et al. (2001), Kurkure et al. (2001), Thenmozhi and Malmarugan (2013) and Durairajan et al. (2013). The

antibiotic resistance of *A. paragallinarum*in this study might be resulted from indiscriminate and inappropriate use of antibiotics.

CONCLUSION

Data of this study indicate that antibiotic resistant *A. paragallinarum* is prevalent in the study area which underscores the need of implementation of effective prevention and control programs through appropriate vaccines, antibiotics and biosecurity measures. This study first report the identification of *A. paragallinarum* from broiler chicken in Bangladesh.

CONFLICT OF INTEREST

Nothing to declare.

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