Isolation and characterization of *Avibacterium paragallinarum* from layer and broiler chickens in Bangladesh

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Abstract

Infectious coryza (IC) is a bacterial disease of the upper respiratory tract of chickens caused by *Avibacterium paragallinarum*. The present study was conducted to investigate the prevalence of *A. paragallinarum* infection in layer and broiler chickens. Detection of *A. paragallinarum* was performed primarily on the basis of isolation, culture and colony characteristics. Nasal and ocular discharges (n=22), tracheal swab (n=15), tracheal washing (n=8), infraorbital sinus exudates (n=10), were collected aseptically from layer (n=43), broiler (n=12) chickens manifested the clinical signs characteristics of IC. The samples were cultured onto blood agar and chocolate agar. Identification of *A. paragallinarum* was performed by Gram’s staining, sugar fermentation and biochemical tests. Antibiotic susceptibility of the bacterial isolated was tested by disk diffusion method. Out of 55 samples tested only four bacterial isolates were confirmed as *A. paragallinarum*. The overall prevalence of IC was 7.27%. Antibiotic susceptibility study indicated that these isolates were sensitive to ciprofloxacin, azithromycin and gentamicin and resistant to ampicillin and cefalexin. Data of this study suggest that multidrug resistant *A. paragallinarum* infection causes outbreak of upper respiratory disease in the layer and broiler birds which underscore the need for implementation of effective prevention and control measures against this disease.

Introduction

*Avibacterium paragallinarum*, a Gram-negative bacterium is responsible for causing respiratory disease of chicken characterized by nasal discharge, conjunctivitis, sinusitis, dyspnea and open mouth breathing. The disease condition caused by *A. paragallinarum* is commonly known as infectious coryza (IC) which mainly affects the nasal passage of chicken (Hoerr et al., 1994). It can affect both young and adult chickens. It is an economically important disease of commercial poultry throughout the world (Gayatri, 2010). Condemnation of poultry carcass due to IC leads to huge economic losses in poultry industry (Droual et al., 1990). The egg production is reduced by 10-40% in the IC affected layer flock and retardation of growth is observed in chickens (Sakamoto et al., 2013). The *A. paragallinarum* normally found in the upper respiratory tract of chickens which can cause disease in chicken under stressful condition. Bird infected with *Pasteurella multocida*, chronic respiratory disease (CRD) and respiratory viruses (Newcastle disease virus, influenza virus) may also be co-infected with *A. paragallinarum* (Reid & Blackall, 1984; Giurov, 1984). The major outbreaks of IC in the commercial poultry farm occur during winter months (Yamamoto et al., 1991). The overcrowding and keeping of multi age chicken in the poultry farm are predisposing factors of the outbreak of IC in chicken (Blackall, 1999). The mortality rate due to IC in chicken range from 1-15% (Sarhiland et al., 2003). The disease is transmitted by air droplets, contaminated feed and water and direct contact with infected birds. This disease is limited primarily to chickens and has no public health significance (Yamamoto et al., 1991).

Several outbreak of IC were reported in the poultry flock in Bangladesh (Talha et al., 2001, Akthar et al., 2001; Akter et al., 2013; Akter et al., 2014). Despite use of antibiotic and vaccines frequent outbreak of the disease is still occur in the poultry in Bangladesh. Development of drug resistance bacteria and change of antigenicity of the causal agent can cause treatment and vaccination failure. In order to undertake appropriate prevention and control measure against IC in chicken detection and characterization of the causal agent from field outbreak is essential. Several outbreaks of IC in two northern and Mymensingh districts of Bangladesh were reported. The present study was undertaken to investigate the outbreak of IC in chicken with the following objectives: (i) isolation of *A. paragallinarum* from sick and dead bird (ii) determination of
prevalence of IC in broiler and layer flocks and (iii) recording antibiotic susceptibility profiles of A. paragallinarum field isolates.

Materials and methods

Birds and study area.
Sick and dead birds were collected from field outbreak of IC in poultry farms located at Rajshahi, Natore and Mymensingh districts of Bangladesh. The details information of outbreak is presented in Table 1. Clinical signs manifested by affected birds were recorded.

<table>
<thead>
<tr>
<th>Study areas</th>
<th>Type of farms affected (n)</th>
<th>No. of bird collected from outbreak</th>
<th>Outbreak season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natore</td>
<td>Layer farms (9) Broiler farms (3)</td>
<td>15 3</td>
<td>Winter</td>
</tr>
<tr>
<td>Rajshahi</td>
<td>Layer farms (18) Broiler farms (5)</td>
<td>25 7</td>
<td>Winter</td>
</tr>
<tr>
<td>Mymensingh</td>
<td>Layer farms (2) Broiler farms (2)</td>
<td>5 0</td>
<td>Winter</td>
</tr>
</tbody>
</table>

Table 1. Detail information of the Infectious coryza outbreak in broiler and layer farms in the study area.

Collection of samples from live birds and dead birds
Nasal, ocular and tracheal swabs were aseptically collected from live birds using sterile cotton swabs. Swab from trachea and infraorbital sinus were aseptically collected from dead birds.

Enrichment of samples
Clinical specimens collected from live and dead birds were enriched in NAD added glycerol-phosphate buffer saline by incubation at 37°C for 24 hrs (Byarugaba et al., 2013).

Isolation 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°C for 24 hrs. Well isolated single colony was further sub-cultured until pure culture was obtained.

Identification of bacteria
Identification of bacteria in pure culture 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 profile of A. paragallinarum. The antibiotics susceptibility testing was performed by disc diffusion method (Bauer et al., 1966). The interpretations of the results were done according to a standard guideline (CLSI, 2007).
Results

Prevalence of A. paragallinarum in chicken
Among 55 birds, clinical samples of 4 birds were culturally positive and the overall prevalence of A. paragallinarum in this study was 7.2% (Table 2).

<table>
<thead>
<tr>
<th>No. of bird tested</th>
<th>No. of positive birds</th>
<th>No. of chicken found positive (%)</th>
<th>No. of negative birds</th>
<th>Overall Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer</td>
<td>Broiler</td>
<td>Layer Broiler</td>
<td>Layer Broiler</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>4</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of A. paragallinarum in chicken.

Prevalence of A. paragallinarum in the study areas
The prevalence of A. paragallinarum in Natore, Rajshahi and Mymensingh was 5.55%, 9.38% and 0% respectively (Table 3).

<table>
<thead>
<tr>
<th>Study area</th>
<th>No. of bird tested</th>
<th>No. of positive bird</th>
<th>No. of negative bird</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natore</td>
<td>18</td>
<td>1</td>
<td>17</td>
<td>5.55</td>
</tr>
<tr>
<td>Rajshahi</td>
<td>32</td>
<td>3</td>
<td>29</td>
<td>9.38</td>
</tr>
<tr>
<td>Mymensingh</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Prevalence of A. paragallinarum in chicken according to study area.

Bacteriological findings
Among 55 samples, bacteria was recovered from 4 samples. Results of colonial morphology of bacteria indicated that all four isolates were A. paragallinarum. The colony characteristics of bacteria recovered from clinical specimen is summarized in Table 4.

<table>
<thead>
<tr>
<th>Name of culture media</th>
<th>Colony characteristics observed on culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate agar with NAD</td>
<td>Dewdrop colonies were observed</td>
</tr>
<tr>
<td>Chocolate agar with feeder organisms (S. aureus)</td>
<td>No growth was found after 48hrs of incubation</td>
</tr>
<tr>
<td>Chocolate agar with NAD &amp; feeder organism (S. aureus)</td>
<td>Dewdrop like colonies throughout the plate were seen</td>
</tr>
<tr>
<td>Chocolate agar without NAD or feeder organism</td>
<td>No growth was found</td>
</tr>
<tr>
<td>Blood agar with feeder organism (S. aureus) and NAD</td>
<td>Characteristic dew drop satellite colonies were found adjacent to the feeder colony</td>
</tr>
<tr>
<td>Blood agar with NAD</td>
<td>Dewdrop colonies were observed</td>
</tr>
<tr>
<td>Blood agar without NAD or feeder organism</td>
<td>No growth was found on blood agar after 48hrs of incubation</td>
</tr>
</tbody>
</table>

Table 4. Cultural characteristics of bacteria grown on the media.

NAD= Nicotinamide adenine dinucleotide.
In Gram’s staining organisms were seen as Gram negative, small rod or cocco-bacilli arranged singly. All the culture positive isolates were found to be non-motile. All culture positive isolates fermented four sugars such as glucose, sucrose, maltose and mannitol and produced only acid without gas. They did not ferment lactose. All isolates were MR-VP, indole and catalase negative. On the basis of results of sugar fermentation and biochemical tests the four isolates were identified as A. paragallinarum. Summary of sugar fermentation and biochemical tests is furnished in Table 5.
Results of sugar fermentation and Results of biochemical tests

<table>
<thead>
<tr>
<th>DX</th>
<th>ML</th>
<th>L</th>
<th>S</th>
<th>MN</th>
<th>Indole</th>
<th>MR</th>
<th>VP</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>-</td>
<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5. Summary of sugar fermentation and biochemical tests.

DX = Dextrose; L = Lactose; S = Sucrose; MN = Mannitol; ML = Maltose; MR = Methyl red; VP = Voges Proskauer; A = Acid; - = Negative.

Antibiotic susceptibility test.
Antimicrobial sensitivity test was performed by disk diffusion method against five antibiotics. All isolates were sensitive to Gentamicin, Ciprofloxacin, and Azithromycin but resistant to Ampicillin and Cefalexin. The result of antimicrobial sensitivity assay is presented in Table 6.

<table>
<thead>
<tr>
<th>Name of antibiotics</th>
<th>Diameter of zone of inhibition (mm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>30</td>
<td>S</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>22</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>31</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>R</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>-</td>
<td>R</td>
</tr>
</tbody>
</table>

Table 6. Antimicrobial sensitivity assay of Avibacterium paragallinarum.
S = sensitive; R = resistant, - = no zone of inhibition.

Discussion
The present investigation was aimed for isolation and identification of A. paragallinarum, the causal agent of IC, from infected chickens during a field outbreak. In this study, chickens of the IC affected farm manifested the clinical signs of ocular and nasal discharges, conjunctivitis, facial swelling and open mouth breathing. The decreased of egg production was also noticed. Similar findings were also reported by other investigators (Amonsin et al., 1997; Haunshi et al., 2006). A study conducted in Egypt Ibrahim et al. (2004) observed severe respiratory signs, and decreased egg production (by 3-40%) in layer chickens.

The bacteriological analysis was performed on samples obtained from 55 chicken which confirmed four isolates of chicken as A. paragallinarum. The rest of the samples were culture negative, although these samples were collected from birds manifested with clinical signs of respiratory diseases. There are several microbial agents also known to cause upper respiratory infections in chicken similar to the signs to IC such as Pasteurella multocida and Mycoplasma gallisepticum and Pseudomonas aeruginosa (Giurov et al., 1984). Treatment of infected chickens with antibiotics at the onset of diseases outbreak by the veterinarian might be also responsible for not isolation of A. paragallinarum from 51 clinically infected chickens.

In the present study A. paragallinarum were recovered from ocular, nasal and tracheal swabs using blood agar and chocolate agar media. Scattered dewdrop like colony without hemolysis was seen in the blood agar plate enriched with NAD. Similar cultural characteristics of A. paragallinarum were reported by Akter et al. (2013) and Akter et al. (2014). In blood agar, dewdrop satellite colonies were seen adjacent to the feeder colony (S. aureus) which were also reported by several investigators (Blackall et al., 1989; Sameera et al., 2001; Christensen et al., 2009; Chukiastsiri et al., 2010).

In the present study, A. Paragallinarum isolates exhibited multidrug resistant profile since they were found resistant to Ampicillin and Cefalexin. However, they were sensitive to Ciprofloxacin, Azithromycin and Gentamicin. Almost similar antibiogram profiles were also recorded by Sameera et al. (2001), Kurkure et al. (2001) and Haunshi et al. (2006). The antibiotic resistance of A. paragallinarum in this study might be resulted from indiscriminate and inappropriate use of antibiotic in the poultry production system.
Conclusion
Data of this study indicate that multidrug resistant A. paragallinarum is prevalent in the study area which underscores the need of implementation of effective prevention and control programs through use of appropriate antibiotics, effective vaccines, and bio-security measures.

References


