Isolation and Identification of Bacterial Flora from Internal Organs of Broiler and Their Antibiogram Studies

Sampa Rani Roy1, Md. Bahanur Rahman1, Jayedul Hassan1 and K. H. M. Nazmul Hussain Nazir2,∗

1Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
2School of Biotechnology, Yeungnam University, Gyeongsan 712-749, South Korea

*Corresponding author’s e-mail: nazir@bau.edu.bd

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A B S T R A C T

The present research work was carried out for the isolation and identification of bacterial flora from internal organs of broiler during the period from January 2012 to June 2012. Ten Hubbard classic broiler bird were purchased from retail market in Mymensingh, Bangladesh. The birds were sacrificed and their liver, lung, esophagus, duodenum and tracheal swab samples were collected (n=50). Using standard bacteriological techniques, Escherichia coli was isolated from 26 (52%) samples. Similarly, Salmonella spp., Staphylococcus spp., Bacillus spp., and Pasteurella spp. were isolated from 15 (30%), 10 (20%), 9 (18%) and 4 (8%) samples, respectively. On the basis of individual sample type, E. coli could be isolated from 8 (80%) duodenum samples being the most prevalent organism. On the other hand, Salmonella spp., Staphylococcus spp., Bacillus spp. and Pasteurella spp. were identified in 5 (50%) lungs, 5 (50%) liver, 4 (40%) duodenum and 2 (20%) lungs samples, respectively. Among these isolated bacteria, E. coli was found to be pathogenic for mice. Antibiogram studies revealed that Ciprofloxacin was highly sensitive against all the isolated bacteria. Diversified bacterial species are prevalent in broiler. However, E. coli and Salmonella spp. infection might make the bird vulnerable for easy access of infection. Proper vaccination and use of selective antibiotics are crucial in protecting broilers from these pathogens.

Keywords: Broiler, internal organ, normal flora, antibiogram

Introduction

Bangladesh is an agriculture based country. As such poultry rearing is considered superior to the others in agricultural sector because of an almost assured in a relatively short period of time. Commercial poultry industry (mostly broilers and layers) plays an important role in the economy of Bangladesh. But the advancement of poultry industry is being hampered by various pathogenic bacterial infections causing nearly 30% mortality of chickens that has been estimated to cost about Tk. 8,000 crores annually in Bangladesh. The bacterial count in poultry housing systems is particularly high in comparison to those of pig and cattle. These pathogens get access to poultry flocks from various sources. Little is known about the bacterial presence in the poultry environment such as in poultry litter and in the poultry house air (Saleh et al., 2003). Intestinal bacteria play an important role on health through their effects on gut morphology, nutrition, pathogenesis of intestinal diseases and immune responses (Mead, 2000). Various pathogenic microbes, such as Escherichia coli, Salmonella spp., Bacillus spp., Streplococcus spp. and Staphylococcus spp., have been implicated to reduce the growth of poultry (Duke, 1986). Broiler is a major fast growing source of meat in the world today. The modern poultry industry can produce market-ready broiler chickens in less than six weeks. This accomplishment has been achieved through genetic selection, improved feeding and keen health management practices including usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems (Apata, 2009). Resistance against frequently used antibiotics has been observed in bacteria present in poultry since the introduction of these antimicrobial agents in poultry. The rise in antibiotic resistance has been reported in the past two decade in many countries including Bangladesh (Kapil, 2004). In Bangladesh, the economic aspect of poultry disease and their mortality and morbidity due to bacterial infection is a matter of great concern to the livestock owners. The antibiotic resistance pattern increases the incidence of disease in poultry and subsequently affects the economy of Bangladesh. Therefore, this study was designed to isolate and identify the associated bacteria prevalent in internal organs of broiler birds and to find out the effective antibiotics against the bacteria through antibiogram studies.

Materials and Methods

Collection of Samples

The research work was conducted during the period from January 2012 to June 2012. Ten Hubbard classic broiler birds were collected from retail marker in Mymensingh, Bangladesh. The birds were sacrificed and the liver, lung, esophagus, duodenum and tracheal swabs were collected aseptically. The collected samples were brought to the Bacteriology Laboratory of the Department of Microbiology and Hygiene under Faculty of Veterinary Science, Bangladesh Agricultural University (BAU) for microbiological analysis.

Isolation of associated bacteria

Primary culture was performed in Nutrient agar and Nutrient broth media. For sub-culturing, suspected bacteria were inoculated separately onto different bacteriological agar media.
under aseptic condition and incubated at 37°C for 24 hours. Pure cultures were achieved as per procedures described by OIE (2000), Merchant and Packer (1967) and Cowan (1985).

Identification of associated bacteria
Cultural, morphological and biochemical characteristics were studied in order to identify the bacterial flora. The cultural characteristics or colonial morphology of the bacteria grown on the nutrient and blood agar media were recorded. Gram staining was performed to study the morphology and staining characteristics of bacteria according to the technique described by Merchant and Packer (1967). Biochemical tests, such as sugar fermentation, coagulase, catalase, MR, VP, and indole tests, were performed per standard methods (Cheshbrough, 1985).

Antibiotic sensitivity test
A previously described disc diffusion process, known as Kirby-Bauer method (Bauer et al., 1966; Jakaria et al. 2012) was used to determine the susceptibility of the bacterial isolates against selected antibiotic agents. For this purpose, eight different antibiotic discs were obtained from commercial sources (Oxoid Ltd, Baring-stoke, Hampshire, England). The selected antibiotics used were Ciprofloxacin (5 µg/disc), Cloxacillin (5 µg/disc), Amoxicillin (10 µg/disc), Chloramphenicol (30 µg/disc), Neomycin (30 µg/disc), Erythromycin (5 µg/disc), Ampicillin (30 µg/disc) and Colistin sulphate (25 µg/disc). The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007; formerly known as NCCLS).

Results
Isolation of bacterial from internal organs of broiler
The bacterial flora isolated from the internal organs of apparently healthy broilers were identified as E. coli, Salmonella spp., Pasteurella spp., Staphylococcus spp. and Bacillus spp. (Table 1).

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Liver (n=10)</th>
<th>Lungs (n=10)</th>
<th>Esophagus (n=10)</th>
<th>Duodenum (n=10)</th>
<th>Tracheal Swab (n=10)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>7 (70)</td>
<td>4 (40)</td>
<td>4 (40)</td>
<td>8 (80)</td>
<td>3 (30)</td>
<td>26 (52)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>3 (30)</td>
<td>5 (50)</td>
<td>4 (40)</td>
<td>3 (30)</td>
<td>NI</td>
<td>15 (30)</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>5 (50)</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td>NI</td>
<td>NI</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>2 (20)</td>
<td>NI</td>
<td>4 (40)</td>
<td>3 (30)</td>
<td>9 (18)</td>
<td></td>
</tr>
<tr>
<td>Pasteurella spp.</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td>NI</td>
<td>1 (10)</td>
<td>NI</td>
<td>4 (8)</td>
</tr>
</tbody>
</table>

Legend: NI- Not identified

Prevalence of bacterial flora in different internal organs of broiler
From the collected samples (n=50), E. coli was isolated from 26 (52%) cases, whereas Salmonella spp. was found in 15 cases (30%). Similarly, the overall prevalence of Staphylococcus spp., Bacillus spp. and Pasteurella spp. found in 10 (20%), 9 (18%) and 4 (8%) samples, respectively. The results have been shown in Fig 1. It is notable that all five different types of bacteria could be isolated from liver. Tracheal swab revealed the presence of only two types of bacteria. No Staphylococcus spp. and Bacillus spp. could be isolated from duodenum and lung samples, respectively. No Bacillus or Pasteurella spp. could be isolated from esophagus samples. E. coli was the most prevalent bacteria in all types of samples in this study (Table 1).

On the basis of individual sample type, the highest prevalence of E. coli (80%) was recorded in duodenum. Similarly, highest prevalence of Salmonella spp. (50%) and Staphylococcus spp. (50%) were found in the lung and liver. On the other hand, the highest prevalence of Bacillus spp. (40%) and Pasteurella spp. (20%) were recorded in duodenum and lung, respectively. The results have been illustrated in Fig 2.

![Fig. 1. Overall prevalence of bacterial flora found in internal organs of broiler. The value indicated for each bar is the overall prevalence of each bacterium.](image)

![Fig. 2. Highest prevalence of bacteria in different internal organs. The value for each bar indicates the highest prevalence of bacteria in respective internal organ.](image)

Antibiogram studies
Based on the susceptibility to antibiotics, the bacteria were categorized into three groups viz. sensitive, intermediate and resistance. Out of eight antibiotics used this study, Ciprofloxacin and Neomycin were found to be sensitive against all five different bacterial isolates. Chloramphenicol was sensitive to four isolates, whereas Ampicillin and Amoxicillin were sensitive to three isolates. Cloxacillin and Erythromycin were found to be sensitive against two bacterial isolates and Colistin sulphate was resistant to four isolates. The antibiotic sensitivity patterns have been summarized in Fig 3.
which is lower than that of the present study. Temelli E. coli duodenum (40%), respectively. Nazir (2011). Considering all the 50 samples, of Wu E. coli Discussion indicating that all were nonpathogenic. Coagulase test was performed using five isolates of Pathogenicity of Legend: P=Pathogenic, NP= Nonpathogenic.

Table 2: Pathogenicity test of E. coli

<table>
<thead>
<tr>
<th>Group</th>
<th>Route of inoculation</th>
<th>Number of mice inoculated</th>
<th>Number of mice died within 48 hrs</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>Oral</td>
<td>2</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>Control</td>
<td>Oral</td>
<td>2</td>
<td>0</td>
<td>NP</td>
</tr>
</tbody>
</table>

Legend: P=Pathogenic, NP= Nonpathogenic.

Pathogenicity of Staphylococcus spp.

Coagulase test was performed using five isolates of Staphylococcus spp. All of the isolates were coagulase negative indicating that all were nonpathogenic.

Discussion

In the present study, five different bacteria (E. coli, Salmonella spp., Pasteurella spp., Staphylococcus spp. and Bacillus spp.) were isolated from the internal organs of broiler. This is in line of findings by Malmuthuge et al. (2012) and Voidarou et al. (2011). Considering all the 50 samples, E. coli was isolated from 26 (52%) samples. This finding is consistent with that of Awad-Alla et al. (2010) and Aguirre et al. (1992) who described a prevalence of 51% in broiler and 52% in black-billed ducks, respectively. The prevalence of Salmonella spp. was 30% in broiler, which is supported by Cardinale et al. (2003), Temelli et al. (2012) and Alcaine et al. (2007). However, a significant variation regarding prevalence of Salmonella spp. was described in other findings, such as 17.9% by Tibaijula et al. (2010) and Aguirre et al. (1992) who described a prevalence of 20%, which is similar to the findings described by Hanning (2012), Alcaine et al. (2009) and Nazir et al. (2005). The E. coli was resistant against Amoxicillin, Erythromycin, Ampicillin, Cloxacillin and Colistin sulphate. Similar findings were reported by Jeyasanta et al. (2012) and Akond et al (2009) and Nazir et al. (2005). The E. coli was resistant against Amoxicillin, Erythromycin, Ampicillin, Cloxacillin and Colistin sulphate. The Pathogenicity test of E. coli in mice Pathogenicity test of E. coli was done by inoculating the bacteria orally in two 1-4 days old mice (Table 2). The experimental mice were died within 48 hrs in the test group, whereas the control mice remained normal. Thus, the isolates were categorized as pathogenic. 

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E. coli was highly prevalent (80%) in duodenum, whereas Salmonella spp. was highly prevalent in lungs (50%). On the other hand, Pasteurella spp., Staphylococcus spp., and Bacillus spp., were found mostly in the lung (20%), liver (50%) and duodenum (40%), respectively. Nazir et al. (2005) studied the prevalence of E. coli in broiler feces and found 65% prevalence which is lower than that of the present study. Temelli et al. (2012) reported a 30.55% prevalence of Salmonella spp. in poultry meat which is lower than that of the present study. In contrast, Hentschel et al. (1979) studied a prevalence of 47% Staphylococcus spp. in cloacal swabs. This variation of results might be due to day by day increasing the load of bacterial flora.

In the present study, E. coli isolated from broiler were found to be sensitive to Ciprofloxin, Chloramphenicol and Neomycin. The results strengthen the earlier observations of Jeyasanta et al. (2012), Akond et al. (2009) and Nazir et al. (2005). The E. coli was resistant against Amoxicillin, Erythromycin, Ampicillin, Cloxacillin and Colistin sulphate. Similar findings were reported by Jeyasanta et al. (2012) and Akond et al (2009). The Salmonella sp. was found to be sensitive to Ciprofloxacin, Amoxicillin, Chloramphenicol and Neomycin. This result was supported by Khan et al. (2005) and De-Jong et al. (2012), where the bacteria were sensitive to Ciprofloxacin and Chloramphenicol. However, our isolates were resistant to Chloramphenicol, Erythromycin, Cloxacillin and Colistin sulphate. The Pasteurella spp. were resistant to Erythromycin and Colistin sulphate but found to be sensitive to Ciprofloxacin, Amoxicillin, Chloramphenicol, Ampicillin, Neomycin and Cloxacillin. Similar results were described by Shivachandra et al. (2004) and Huang et al. (2009). Selleyi et al. (2009) found Pasteurella spp. to be sensitive to Colistin sulphate. A possible cause of this variation could be due to random use of antibiotic resulting resistance against different antibiotics. Among the Gram positive bacteria, Staphylococcus spp. was found to be sensitive to all tested antibiotics. Bacillus spp., was sensitive to Cloxacillin and Colistin sulphate, which supports the findings of Nasrin et al. (2007) and Guven et al. (2005).

The pathogenicity test for all the isolated E. coli revealed that all were pathogenic although the bacteria were originated from apparently healthy birds. Landman and Cornelissen (2006) reported that more than one predisposing factors such as environmental and managerial factors (housing, climate etc), imbalance nutrition and immune status of the poultry might play roles in developing diseases while harboring the potential pathogenic bacteria. The pathogenicity test of Staphylococcus spp. was performed by coagulase test. All of the isolates were coagulase negative and therefore, were considered as non pathogenic. Staphylococcus spp. can be either coagulase positive or coagulase negative (Adegoke, 1986), and Staphylococcus aureus is commonly recognized as the only coagulase positive candidate. Additional research is required for further characterization of the bacterial isolates described in this study.

Conclusion

Escherichia coli, Salmonella spp., Staphylococcus spp., Bacillus spp., and Pasteurella spp. were isolated from internal organ samples of broilers collected from a local retail market in Bangladesh. Prudent use of antibiotics should be considered in broiler production (where permissible) since many strains are resistant to common antibiotics as described in this study. Potential drug resistant pathogens in otherwise normal broilers may be a serious concern for public health. Current findings warrants further studies with the isolated stains of bacteria.

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References


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