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

Sampa Rani Roy[1], Md. Bahanur Rahman[1], Jayedul Hassan[1] and K. H. M. Nazmul Hussain Nazir[1,2*]

[1]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]School 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., *Staphylococci* 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., *Streptococcus* 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.

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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^{0}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 (Cheesbrough, 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 1: Bacterial flora isolated from internal organs (n=50) of broiler

Isolated bacteria	No. of positive samples and prevalence (%)					Total (%)
	Liver (n=10)	Lungs (n=10)	Esophagus (n=10)	Duodenum (n=10)	Tracheal Swab (n=10)	
E. coli	7 (70)	4 (40)	4 (40)	8 (80)	3 (30)	26 (52)
Salmonella spp.	3 (30)	5 (50)	4 (40)	3 (30)	NI	15 (30)
Staphylococcus spp.	5 (50)	3 (30)	2 (20)	NI	NI	10 (20)
Bacillus spp.	2 (20)	NI	NI	4 (40)	3 (30)	9 (18)
Pasteurella spp.	1 (10)	2 (20)	NI	1 (10)	NI	4 (8)

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.

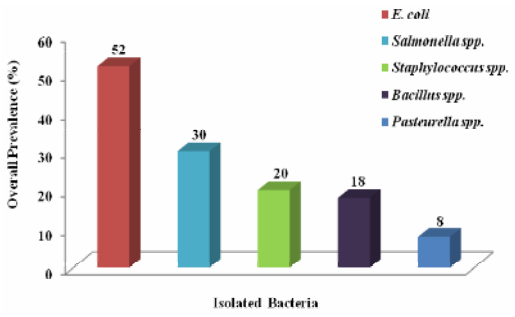

Fig. 1. Overall prevalence of bacterial flora found in internal organs of broiler. The value indicated for each bar is the overall prevalence of each bacteium.

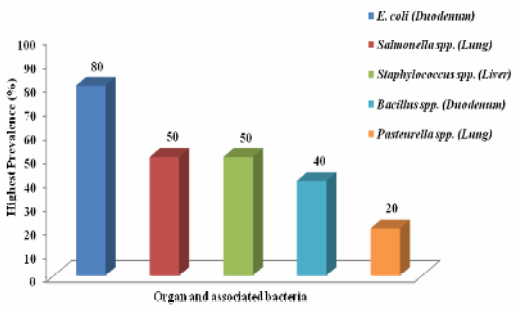

Fig. 2. Highest prevalence of bacteria in different internal organs. The value for each bar indicates the highest prevalence of bacteria in respective internal organ.

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.

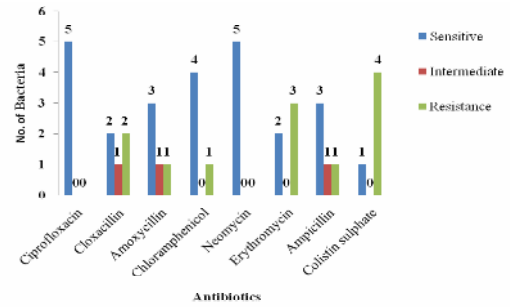

Fig. 3: Antibiogram studies against the isolated bacteria. The value for each bar indicates the number of isolate(s) showing sensitivity against respective antibiotic.

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.

Table 2: Pathogenicity test of *E. coli*

Group	Route of inoculation	Number of mice inoculated	Number of mice died within 48 hrs	Inter-pretation
Test	Oral	2	2	P
Control	Oral	2	0	NP

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*. (2003), 14.37% by Petrovic *et al.* (2011) and 13% by Ellerbroek *et al.* (2010). Similarly, Afroz et al. (2012) reported a 26.02% prevalence of *Salmonella* spp. in internal organs of layer. These variations might be due to difference in sample size, geographical location and type of bird. In this study, the prevalence of *Pasteurella* spp. was 8%. Similar observation was also recorded by Spadafora *et al.* (2011) and Tatum *et al.* (2005). Among the Gram positive bacteria, overall prevalence of *Staphylococcus spp.* was 20%, which is similar to the findings described by Hanning *et al.* (2012) and Alfonso and Barnes (2006). The overall prevalence of *Bacillus spp.* was 18%, which supports the findings of Wu *et al.* (2011) and Hoq *et al.* (2005).

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 Ciprofloxacillin, Amoxicillin, Chloramphenicol, Ampicillin, Neomycin and Cloxacillin. Similar results were described by Shivachandra *et al.* (2004) and Huang *et al*. (2009). Sellyei *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 managemental 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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