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Multidrug Resistant Bacteria in the Respiratory Tract of Apparently Healthy Quails

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A B S T R A C T

The study was undertaken for isolation, identification and *in vitro* antibiotic sensitivity assay of bacteria present in the respiratory tract of apparently healthy Japanese quails. A total of 50 samples comprised of tracheal swabs (n = 18), tracheal washings (n = 8), air sacs (n = 8), lungs (n = 8) and exudates of infraorbital sinuses (n = 8) were aseptically collected from 26 apparently healthy Japanese quails. The samples were inoculated onto a variety of media for isolation of bacteria. Identification of bacteria was performed by colony morphology, Gram's staining and biochemical tests. In total, 25 (50%), 9 (18%), 22 (44%), 20 (40%) and 24 (48%) isolates were identified as *Escherichia coli*, *Salmonella* spp., *Pasteurella* spp., *Bacillus* spp. and *Staphylococcus* spp. respectively. Antibiotic sensitivity tests of one randomly selected isolate from each genus of bacteria were performed against 10 common antibiotics. *E. coli* showed resistance to four antibiotics (amoxicillin, gentamycin, nalidixic acid and tetracycline); *Salmonella* spp. showed resistance to seven antibiotics (amoxicillin, ampicillin, erythromycin, gentamycin, kanamycin, nalidixic acid and tetracycline); *Pasteurella* spp. to three antibiotics (erythromycin, sulphamethoxazole and tetracycline); *Bacillus* spp. to chloramphenicol and nalidixic acid and *Staphylococcus* spp. to amoxicillin and ampicillin. All the isolates were susceptible to ciprofloxacin. Data of this study indicate that multidrug resistant bacteria are present in the respiratory tract of clinically healthy quails. This is the first report of isolation, identification and antibiogram profile of bacteria present in the respiratory tract of quails in Bangladesh.

Keywords: Multidrug resistant bacteria, antibiotic sensitivity, respiratory tract, quails

Introduction

Respiratory diseases are one of the important health problems in all species of domestic birds. A variety of clinical signs are seen in quails with bacterial respiratory diseases such as: weakness, dyspnea, nasal discharge, facial edema, swelling of sinuses and increased mortality (Murakami *et al.,* 2002; Thenmozhi *et al.,* 2010). Both Gram-positive and Gram-negative normal bacterial flora have been isolated from the respiratory tract of birds such as: *Staphylococcus aureus, Corynebacterium* spp., *Erysipelothrix* spp., *Klebsiella* spp., *E. coli, Pseudomonas* spp. and *Pasteurella* spp. (Sambyal and Baxi, 1980). There are many factors that predispose the respiratory tract infections by the normal bacterial flora. Diet deficient of vitamin A results in abnormal epithelium of respiratory tract, which could be easily infected by bacteria. Use of immunosuppressive drugs, poor host immunity and viral infection also enhance the susceptibility of respiratory tract infection in birds, often by low virulent normal inhabitant bacteria of the respiratory tract (Nakamura *et al.,* 1992). Quail production is constantly facing threat of emerging bacterial diseases such as: salmonellosis, collibacillosis, infectious coryza, ulcerative enteritis and staphylococcosis (Kenneth, 2008). *Pasteurella* spp., *E. coli*, *Streptococci* and *Staphylococci* were isolated from the oculofacial respiratory diseases of quails along with M*ycoplasma gallisepticum* (Murakami *et al.,* 2002).

Identification of bacteria present in the respiratory tract of quails and to know their antibiotic sensitivity assay are essential for undertaking effective prevention and control measures against the bacterial infections. To the best of our knowledge, there is no report on the isolation, identification and antibiotic sensitivity profiles of bacteria present in the respiratory tract of quails in Bangladesh. The objectives of the present research work were to isolate and identify bacteria from the respiratory tract of apparently healthy quails and to determine their antibiotic sensitivity profiles against common antibiotics.

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Materials and Methods

Collection of samples

A total of 50 samples such as: tracheal swabs (n =18), tracheal washings (n = 8), air sacs (n = 8), lungs (n = 8), infraorbital sinuses (n = 8) were aseptically collected from 26 apparently healthy Japanese quails without manifesting any clinical sign of illness at Bangladesh Agricultural University (BAU) poultry farm during the period from January 2011 to June 2011. The samples were transported to the laboratory at 4°C.

Isolation of bacteria

The samples were enriched in nutrient broth by overnight incubation at 37^0C. A loopful of enrichment culture was streaked onto nutrient agar, blood agar, salmonella-shigella (SS) agar, manitol salt (MS) agar, brilliant green (BG) agar and eosine-methylene blue (EMB) agar and incubated at 37 ^{0}C overnight for isolation of bacteria.

Identification of bacteria

Colony morphology of bacteria such as: shape, size, surface texture, edge, elevation and color observed on pure culture, Gram staining and biochemical tests (sugar fermentation, methyl red, Voges-Proskauer and indole production tests) were used for

identification of bacteria (Cheesbrough, 1985; Buxton and Fraser, 1977).

Antimicrobial susceptibility testing

The disk diffusion method (Bauer *et al.,* 1966) was used to test antimicrobial susceptibility of the bacterial isolates of quails using 0.5 McFarland turbidity standard inoculum and freshly prepared dried Mueller Hinton agar (Oxoid, UK). One isolate from each genus of bacteria was selected randomly for antibiotic sensitivity test against 10 antimicrobial discs (Oxoid, UK). Antimicrobial agents and their disc concentrations used were as follows: ampicillin (10 µg), amoxicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), sulphamethoxazole (25 µg), tetracycline (30 µg) and nalidixic Acid (30 µg). Results of antibiotic sensitivity tests were recorded as sensitive, intermediate and resistant following the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007).

Results

Bacteriological findings

The bacterial isolates were identified as *E. coli, Salmonella* spp*., Pasteurella* spp., *Bacillus* spp. and *Staphylococcus* spp. according to the results of cultural characteristics, colony morphology, Gram's staining, sugar fermentation reaction and biochemical tests. Summary of cultural characteristic, sugar fermentation and biochemical tests are presented in Table 1 and Table 2, respectively.

Prevalence of bacteria in the respiratory samples

In total, 25 (50%) *E. coli*, 9 (18%) *Salmonella* spp., 22 (44%) *Pasteurella* spp., 20 (40%) *Bacillus* spp. and 24 (48%) *Staphylococcus* spp. were recovered from the respiratory samples of quails. Summary of bacteria recovered from various respiratory samples of quails are presented in Table 3.

E. coli prevalence rate was the highest in tracheal swabs (83.33%) followed by air sacs (75%), lungs (62.5%), infraorbital sinuses (50%) and tracheal washings (37.5%) of quails less than one month of age.. *Salmonella* spp. were not recovered from tracheal swabs, air sacs and infraorbital sinuses of quails in that age group. However, prevalence rates of *Salmonella* spp. were 12.5% both in tracheal washings and lungs. The prevalence rate of *Pasteurella* spp. was the highest in tracheal swabs (66.67%), followed by lungs (62.5%), infraorbital sinuses (37.5%), tracheal washings (25%) and air sacs (12.5%). The highest prevalence rate of *Bacillus* spp. was in lungs (87.5%) followed by tracheal washings (50%), air sacs (37.5%), infraorbital sinuses (25%) and tracheal swabs (16.67%). Prevalence rate of *Staphylococcus* spp. was the highest in lungs (75%), followed by tracheal swabs (33.33%), tracheal washings and air sacs (25% and 25%) and infraorbital sinuses (12.5%). The prevalence rate of bacteria in different respiratory samples of young quails under one month of age is shown in Fig. 1.

Prevalence of bacteria recovered from tracheal swabs was compared between less than one month and six months old quails. Prevalence rates of *E. coli*, *Salmonella* spp*., Pasteurella* spp*., Bacillus* spp. and *Staphylococcus* spp. were 16.67%, 58.33%, 50%, 25% and 91.67%, respectively in the tracheal swabs of six months old quails.

Antibiotic sensitivity assay

All the bacterial isolates were resistant to at least two antibiotics and sensitive to only ciprofloxacin. *Salmonella* spp. showed resistance to seven antibiotics, *E. coli* showed resistance to four antibiotics and *Pasteurella* spp. to three antibiotics. Both *Bacillus* spp. and *Staphylococcus* spp. were resistant against two antibiotics. The summary of antibiotic sensitivity assay is presented in Table 4.

Discussion

Respiratory infection causes heavy economic losses in the poultry industry worldwide (Murthy *et al.,* 2008). Bacterial pathogens play an important role in causing respiratory disease in poultry species. In many cases, the bacterial component colonizes the respiratory system only after a primary viral or environmental insult. Colonization of the air sacs of a chicken by *E. coli* following an infectious bronchitis virus infection is an example of secondary bacterial invasion. In other cases, the bacterial component of the respiratory disease is the primary initiating cause of the disease. Examples of primary bacterial respiratory disease are infectious coryza in chickens and fowl cholera in chickens and turkeys (Glisson, 1998). In avian hosts, several microorganisms of the genus *Pasteurella, Bordetella* and *Haemophilus* were involved in respiratory diseases (Hafez, 2002). *E. coli* associated with respiratory infection in chickens have also been reported (El-Sukhon*et al.,* 2002). *Salmonella* spp*., Bacillus* spp. and *Staphylococcus* spp. are considered as opportunistic bacteria in the respiratory tract.

In this study, *E. coli, Salmonella* spp*., Pasteurella* spp*., Bacillus* spp. and *Staphylococc* spp. were isolated from the respiratory tract of the apparently healthy quails. Isolation of bacteria from respiratory tract of healthy quails indicated that these are the normal flora in this species. Although normal bacterial floras are generally harmless, they can cause diseases when the host defenses are impaired (Macowicak, 1982). Thenmozhi *et al.* (2010) identified *E. coli*, *Ornithobacterium rhinotracheale*, *P. multocida* and *Haemophilus paragallinarum* in the respiratory tract of chickens suffering from respiratory diseases. *P. multocida*, *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp. have been isolated from the infraorbital sinuses of quails with signs of respiratory illness (Murakami *et al*., 2002). Thenmozhi *et al.* (2010) isolated *E. coli, P. multocida,* and *H. paragallinarum* from the Japanese quails suffering from respiratory tract diseases. *S. typhimurium* were isolated from the lungs of macaw chicks (Vigo *et al.,* 2009) and *Bacillus* was isolated from the lung of the psittacine birds in a zoo of Brazil (Godoy *et al.,* 2012).

In the present study, out of 50 samples, 25 were positive for *E. coli* (50%), 9 were positive for *Salmonella* spp. (18%), 22 were positive for *Pasteurella* spp. (44%), 20 were positive for *Bacillus* spp. (40%) and 23 were positive for *Staphylococcus* spp. (46%). Thenmozhi *et al.* (2010) isolated 56.14% *E. coli* and 28.07% *Pasteurella* spp. from respiratory samples of birds. A study conducted at the Tamil Nadu state in India isolated 51.9% *E. coli* and 9.6% *Pasteurella* spp. from the birds with respiratory diseases (Murthy *et. al.,* 2008).

Randomly selected one isolate of each genus of bacteria recovered from quails in the current study showed multidrug resistance profile. In this study, *Salmonella* spp. showed resistant profile against seven antibiotics such as: amoxicillin, ampicillin, erythromycin, gentamycin, kanamycin, nalidixic acid and tetracycline. Multidrug resistant *Salmonella* spp. have been emerging worldwide in the recent years (Zhanng *et al.,* 2005). A study conducted in Denmark found that *Salmonella* spp. isolated from humans, animals, food products and environmental samples were resistant to ampicillin, chloramphenicol, nalidixic acid, sulphamethoxazole and susceptible to ciprofloxacin and gentamycin (Aarestrup *et al.,* 2003). *E. coli* isolate of quail in this study showed resistant to four antibiotics namely, amoxicillin, gentamycin, nalidixic acid and tetracycline. *E. coli* isolates of chickens in Iran were found resistant against tetracycline, amoxicillin and ampicillin (Tabatabaei *et al.,* 2010). *Pasteurella* spp. isolated from quails in the present study showed resistance against erythromycin, sulphamethoxazole and tetracycline. Our findings contradict the finding of Morishita *et al.* (1996) who reported that *P. multocida* isolates were susceptible to erythromycin, sulphamethoxazole and tetracycline. Sellyei *et al.* (2009) reported that *P. multocida* isolates of poultry were resistant to sulphonamide and tetracycline. *Bacillus* sp. showed resistance to chloramphenicol and nalidixic acid. Barbosa *et al.* (2005) observed that *Bacillus* spp. of broilers were resistant to

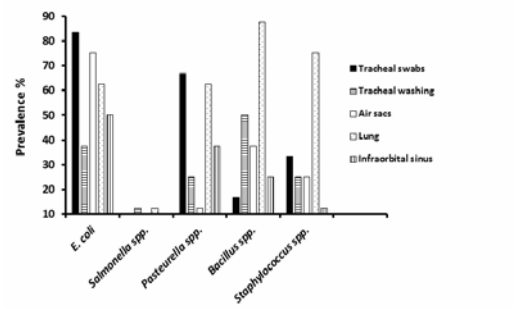

Fig. 1. Prevalence of *E. coli*, *Salmonella* spp., *Pasteurella* spp., *Bacillus* spp. and *Staphylococcus* spp. in tracheal swabs, tracheal washings, air sacs, lungs and infraorbital sinuses of quails less than one month of age

Table 1. Cultural characteristics of the bacteria isolated from respiratory samples of quails

Cultural characteristics of bacteria isolated from quails on					Interpretation
Nutrient agar	**Blood agar**	**MacConkey agar**	**EMB agar**	**SS agar**	
Smooth, circular, white to grayish white colony	Circular raised colony with no hemolysis	Rose pink, circular raised colony which were lactose fermenter	Moist circular colony with dark centers yellow green metallic sheen	Pink colored, round, smooth colony	*E. coli*
Circular smooth, raised colony	White, round, raised colony with no hemolysis	Pale, transparent, circular, smooth raised colony	No growth	Opaque, translucen, round, smooth colony	*Salmonella* spp.
Thick grayish white or cream colored circular colony	Abundant growth. Creamy yellow colored colony and hemolysis	No growth	No growth	No growth	*Bacillus* spp.
Gray white or yellowish color round, smooth colony	White to golden yellow colony with no hemolysis	No growth	No growth	No growth	*Staphylococcus* spp.
Whitish, opaque, circular, translucent appearance	Whitish, opaque, circular, translucent appearances and no hemolysis	No growth	No growth	No growth	*Pasteurella* spp.

EMB = Eosine methylene blue; SS = Salmonella- shigella agar

Table 2. Results of biochemical tests of the bacteria isolated from respiratory samples of quails

Fermentation test using					MR test	V-P test	Indole test	Interpretation
Dextrose	**Lactose**	**Sucrose**	**Maltose**	**Mannitol**				
AG	AG	AG	AG	AG	+	-	+	*E. coli*
AG	-	-	AG	AG	+	-	-	*Salmonella* spp.
A	-	A	-	A	-	-	+	*Pasteurella* spp.
AG	A	AG	A	AG	+	-	+	*Bacillus* spp.
A	A	A	A	A	+	-	+	*Staphylococcus* spp.

A = Acid; AG = Acid and gas; + = Positive reaction; - = Negative reaction; MR = Methyl red; V-P = Voges-proskauer

Table 3. Summary of bacterial isolates recovered from the respiratory samples of Japanese quails

Name of samples (n)	Age of birds	Name of bacterial isolates				
		***E. coli* (n)**	***Salmonella* (n)**	***Pasteurella* (n)**	***Bacillus* (n)**	***Staphylococci* (n)**
Tracheal swabs (12)	6 months	2	7	6	3	11
Tracheal swabs (6)	<1 month	5	ND	4	1	2
Tracheal washings (8)	<1 month	3	1	2	4	2
Air sacs (8)	<1 month	6	ND	1	3	2
Lung (8)	<1 month	5	1	5	7	6
Infraorbital sinuses (8)	<1 month	4	ND	3	2	1

ND = Not detected

Table 4. Antibiotic sensitivity profiles of respiratory bacterial isolates of quails

Name of bacteria	Antibiotic sensitivity profiles of bacterial isolates against									
	AML	**AMP**	**C**	**CIP**	**E**	**GN**	**K**	**NA**	**SXT**	**TE**
E. coli	R	S	S	S	S	R	S	R	S	R
Salmonella	R	R	S	S	R	R	R	R	I	R
Pasteurella	S	S	S	S	R	S	S	I	R	R
Bacillus	S	S	R	S	S	S	S	R	S	S
Staphylococci	R	R	S	S	S	S	S	S	S	S

I = Intermediate; R = Resistant; S = Sensitive; AML = Amoxicillin; AMP = Ampicillin; C = Chloramphenicol; CIP = Ciprofloxacin; E = Erythromycin; GN = Gentamycin; K =Kanamycin; NA = Nalidixic acid; SXT = Sulphamethoxazole; TE = Tetracycline

erythromycin. *Bacillus* sp. was found sensitive to erythromycin in this study. *Staphylococcus* sp. of quail in this study showed resistance to ampicillin and amoxicillin. This finding is in agreement with the finding of Klimiene *et al.* (2011) who noticed that *S. aureus* were resistant to ampicillin and amoxicillin.

The widespread use of antibiotics as therapeutic and prophylactic agents and as growth promoters in animal husbandry has led to a worldwide increase in antibiotic resistance and the emergence of multidrug-resistant strains of bacteria (Barbosa and Levy, 2000; Courvalin, 2005). Therefore, judicial uses of antibiotics need to be practiced to minimize the emergence of drug resistant bacteria.

Conclusion

In conclusion, our study identified multidrug resistant Gram-positive and Gram-negative bacteria in the respiratory tract of quails. These multidrug resistant bacteria may get transferred to humans, animals and birds through direct contact or via food chain resulting complications in treatment regimen against these bacterial infections. Data of this study would be helpful for undertaking prevention and control measures against respiratory tract bacterial infections in quails.

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