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Article

Isolation and identification of microorganisms from cloacal swabs in poultry

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Abstract: This study was carried out for the isolation and identification of microorganism from cloacal swabs in poultry. During the period from January 2014 to June 2014, the entire research work was conducted in laboratory of the department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The study was performed with 24 cloacal swabs samples. Samples were collected from the poultry as 6 samples from 5 days of old, 9 samples from 18 days of old and 9 samples from 28 days of old and transferred aseptically to the laboratory. All the experimental work was done under sterile condition. Out of the 24 cloacal swabs samples, 20 isolates were positive. Among 20 positive samples, 6 (30%) *E. coli*, 5 (25%) *Salmonella* spp., 4 (20%) *Staphylococci* spp., 2 (10%) *Mycoplasma* spp. and 3 (15%) fungus were grown in culture media. In gram's staining from ordinary media (nutrient agar), both gram positive and grams negative bacteria were found. Mycoplasma was positive in Mycoplasma agar (PPLO) where color was changed to slight grayish. On sabourauds dextrose agar medium, the organism were grown as round and raised structure which indicated that fungus was present there. Isolation and identification was done by observing colony morphology, grams staining and standard cultural and biochemical test. Diversified bacterial species were prevalent in cloacal swabs of broiler. However *E. coli*, *Salmonella* spp. infection might make the birds vulnerable for easy access of infection. It could be concluded that the organisms may pass through the faeces to the environment and cause a potential human health hazards and can cause illness.

Keywords: cloacal swabs; broiler; isolation and identification; microorganisms

1. Introduction

Bangladesh is an agriculture based country. About 80% people of the country are dependent on agriculture either directly or indirectly. The poultry industry comprising of commercial poultry mainly broiler and layers plays an important role in the economy of Bangladesh. There are approximately 163.50 million of poultry including ducks reared throughout Bangladesh (Giasuddin *et al.*, 2002). 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 through their effects on gut morphology, nutrition, pathogenesis of intestinal diseases and immune responses (Mead, 2000).The rural poultry production system in Bangladesh is typically a smallholder free range scavenging operation. Development of poultry sector in Bangladesh is being hampered by a number of factors, of which the diseases are considered as the major factor causing 30% mortality of chicken per year (Das *et al.*, 2005). Various pathogenic microbes *E. coli*, *Salmonella* spp. *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp., have been implicated to reduce the growth of poultry (Duke, 1986). *E. coli* is a major pathogen of commercially produced poultry causing Colibacillosis all over the world.

E. coli is a Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobes. Salmonellosis is one of the most important diseases that causes serious economic loss due to mortality and reduced egg production (Khan *et al.,* 2005). *Salmonella* are Gram negative, small rod-shaped, non-spore forming, non-capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE, 2000). The salmonella are potentially responsible for various pathogenic processes in man and animal including poultry (Freeman, 1985). Age wise prevalence of avian Salmonellosis showed highest infection rate in adult layer (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) (Rahman *et al.,* 2004). Staphylococcosis an infectious disease caused by staphylococci that affects all bird species. The causative agents are diseased birds. The disease is generally transmitted in feed, litter, and water. It may also be transmitted through transovarially, which is by way of the egg. Acute avian Staphylococcosis in chickens is occurred the clinical sign as diarrhea, depression and inflammation of the joints. Mycoplasmosis, pathogen interactions play the most significant role in development of respiratory disease complex and the Mycoplasma infections are responsible for major losses. The study was carried out to isolate and identify microorganisms from cloacal swab in poultry and to determine the pathogenic microorganisms present as well as their potential transmission and health implications on the human environment.

2. Materials and Methods

2.1. Collection of samples

The research was carried out on different commercial broiler farm at saidpur, Sadar upazila of Nilphamari and Khansama of Dinajpur district. The cloacal swabs sample was collected carefully from the birds during the period from January 2014 to June 2014.The collected samples were brought to the bacteriology laboratory of the department of microbiology under the faculty of Veterinary and Animal Science, Hajee Mohammad Danesh science and Technology University (HSTU), Dinajpur. A total of 24 broiler samples of 5 days, 18 days and 28 days old were included in this study using routine techniques for aseptic infection. Sterile cotton sticks with sterile normal saline was inserted to collect the cloacal contents of chicken for isolation and identification of organism.

2.2. Isolation of bacteria in pure culture

Pure cultures of bacteria obtained by aseptically streaking representative colonies of different morphological types, which appear on the culture plates on to freshly prepared nutrient agar plates from the incubator. Discrete bacteria colonies that developed were subculture on nutrient agar slopes and incubated at 37^{o}C for 24 hours. Pure cultures were achieved as per procedures described by OIE (2000), Merchant and Packer (1967) and Cowan (1985).

2.3. Cultivation of fungi

For the detection of fungi the diluted samples were placed on Sabouraud dextrose agar and incubated at 24^{o}C for 4 to 10 days. Fungi were identified macroscopically based on colony morphology and microscopically using lactophenol staining. Colonies that developed were observed macroscopically for distinguishing characteristics. The complete identification of fungal isolates was by comparing the result of their cultural and morphological characteristics with (Barnett *et al*., 1972).

2.4. 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).

2.4.1. Isolation of *E. coli*

Isolation the organism with supporting growth characteristics of *E. coli* was maintained on EMB agar and was subjected to the biochemical tests named as TSI, MR-VP and Indole reaction. The suspected colony was inoculated into the surface into the surface of the slants and stabbed into the butt of the tubes containing TSI agar. Then the tube was incubated at 37^{o}C for 24 hours. For MR-VP tests, the tube containing 2ml of MR-VP broth was inoculated with the isolated organism. Tubes for VP test were inoculated at 37^{o}C for 24 hours but for 5 days at the same temperature for MR test. To perform VP test 1.2 ml of 5% alkaloid alpha-naphthol and 0.2

ml of 40% KOH were added in each inoculated VP test. The ingredients were mixed thoroughly and kept in still moment. a strip of filter paper was soaked in oxalic acid and dried and then hung in the form of loop over the tryptone broth in a culture tube for Indole test. Secured the ends of the paper that's between the cotton plugged and mouth of the tube.

2.4.2. Isolation of *Salmonella* spp.

Isolation the organism with supporting growth characteristics of *Salmonella* were maintained Salmonella-Shigella agar and subjected to the biochemical test named as TSI, MR-VP, Simmons Citrate agar and Indole test. For the Triple sugar Iron agar Slant test the suspected colony was inoculated into the surface of the slants and stabbed into the butt of tubes containing TSI agar. Then the tube was incubated at 37^{o}C for 24 hours and for the Methyl Red Test a colony of the test organism was inoculating in 0.5ml sterile glucose phosphate peptone broth. After overnight incubation at 37^{o}C a drop of methyl red solution was added. About 2ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organism. It was incubated at 37^{o}C aerobically for 48 hours. A very small amount (Knifepoint) of creative was added and mixed for the Voges-Proskaure Test. Suspected colony was also inoculated on simmons citrate agar. Then the medium was incubated at 37^{o}C for 48 hours for Simmons Citrate agar test.

2.4.3. Isolation of *Staphylococcus* spp.

The Incubation was done by nutrient agar to selective staphylococcus-110 agar medium. Then it's incubated in incubator at 37^{o}C for 24 hours. Grams staining were also performed to determine the size, shape, and arrangement of bacteria. Gram's staining reaction was performed according to the methods described by Cowan (1985). Nutrient agar slants were used for the maintenance of stock for each of the bacterial isolates. One slant was used for an individual isolate. After growth of the organism in the slant, the sterile mineral oil was overlaid and the culture was kept at room temperature for further use as seed.

3. Results

The staining characteristics of the isolated organisms were determined according to Gram's staining technique and the results are presented in Table 1. Smears from EMB and Simmons citrate agar the organism found as gram negative, rods of different shape and size, which arranged singly, pair or in short chain indicating E. *coli*. Gram's stained smears from SS agar revealed gram negative, pink colored, short plump rod shaped appearance, arranged in single and paired indicating *Salmonella* spp. Stained smear of the slide revealed gram positive cocci arranged in cluster indicating *Staphylococcus* spp. are present there.

Table 1. Morphology, staining and motility characteristics of bacterial isolates.

Bacterial Isolates	Shape	Arrangement	Gram's staining reactions	Motility characteristics
E. coli	Rod or coccobacilli	Single or paired	Gram negative	Motile
Salmonella spp.	Small rod	Single	Gram negative	Non Motile
Staphylococcus spp.	Cocci	Cluster, grape like	Gram positive	Motile

The individual culture characteristics of bacterial isolates are also presented in Table 2. The isolate *E. coli* produced smooth, circular, and white to grayish white colony with peculiar fetid odor on nutrient agar and raised moist circular colonies with dark centers on Simmons-Citrate and EMB agar. *Salmonella* spp. colonies are smooth and colorless in Mac-Conkey agar media indicating non-lactose fermenter. *Salmonella pullorum* produces smaller colonies than other *Salmonella*, gray mucoid colony on SS-agar, *Salmonella* form low, convex, pale red, translucent colonies of 1-3 mm in diameter, similar to citrobacter. *Salmonella pullorum* produces smaller more pale colonies than other *Salmonella*. *Staphylococcus* spp. isolated from the cloacal swab revealed following cultural characteristics on selective nutrient agar media. The organisms showed grey-white to yellowish colony on nutrient agar. *Mycoplasma* spp. inoculation on mycoplasma agar, after 24 hours incubation the selective agar media was change into color with slight grayish color and culture is formed. Fungus inoculation on on sebouraued dextrose agar medium, the organism grows in round and raised structure which indicates that fungus is present there. Biochemical test Triple sugar iron agar, methyl red test, Vogesproskauer test, MIU test and buffer peptone water test (Indole test) were to determine their biochemical characters and degree of variation in their reactivity pattern. The result was presented in Table 3. The result of frequency distribution of different bacterial isolates is presented in Figure 1. Out of the 24 cloacal swabs samples, 20 isolates were positive of which *E. coli* was 6 (30%), *Salmonella* spp. 5 (25%), *Staphylococcus* spp. 4 (20%) and *Mycoplasma* spp. 2 (10%) and 3 (15%) fungus were grown in subculture media.

Table 2. Results of cultural characteristics of the organisms which are isolated from cloacal swabs in poultry.

Isolated Organism	Nutrient Agar	EMB Agar	Mac-Conkey agar	SS agar	Satphylococcus -110 agar	SDA	SCA	MPA
Escherichia coli	Smooth, circular, White to grayish white colony	Smooth, Large, circular, blueblack colonies with slightly green metallic sheen	Smooth pinkish colony	Slight growth and pink to rose-red colonies	No growth	No growth	No growth	
***Salmonella* spp.**	Circular, smooth, opaque and translucent	Pink color, circular and smooth colony	Smooth and circular white/transparent colony	Black, smooth, small round colony	No growth	No growth	No growth	
***Staphylococcus* spp.**	Gray white or yellowish colony	No growth	No growth	No growth	The organisms showed grey-white to yellowish colony	No growth	No growth	
***Mycoplasma* spp.**	No growth	No Growth	No growth	No growth	No growth	No growth	No growth	The selective agar media was change into slight grayish color and culture formed.
Fungus and others	No growth	No growth	No growth	No growth	No growth	The organism grows in round and raised structure.	No growth	No growth

Legends: EMB = Eosin Methylene Blue, SS = Salmonella-Shigella, SDA= Sabourauds dextrose agar, SCA= Simmons citrate agar, MPA= Mycoplasma agar,

Table 3. Result of Biochemical tests of the isolated *E. coli, Salmonella* spp. and *Staphylococcus* spp. from cloacal swabs in poultry.

Isolated Bacteria	Indole	MR	VP	TSI			MIU
				Butt	Slant	H2S	
E. coli	+	+	+	**Y**	**Y**	-	+
Salmonella spp.	+	+	-	**Y**	**R**	+	+
Staphylococcus spp.	+	+	+	**Y**	**Y**	-	+

Legends
MR= Methyl Red, VP= Voges-Proskaure, TSI= Triple Sugar Iron, " +"= Positive, "-"= Negative, Y= Yellow, R= Red, Indole= Buffer Peptone water, MIU= Motility, Indole and Urease test.

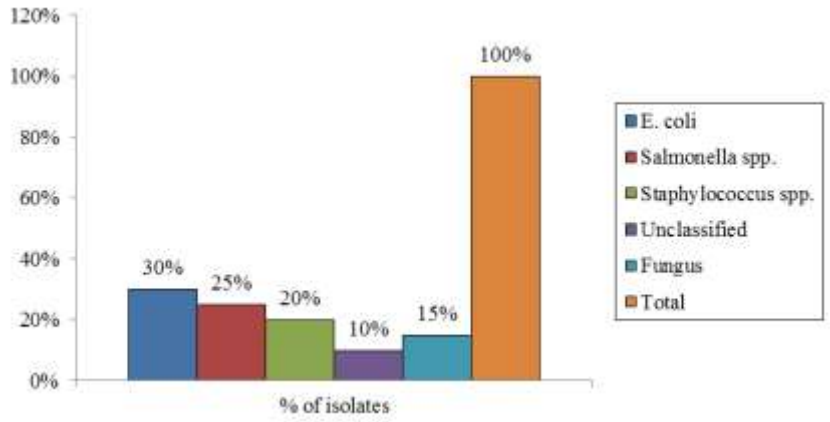

Figure 1. Frequency distribution of organism from cloacal swabs from poultry.

4. Discussion

In this experiment there were three types of organism as like bacteria; Mycoplasma and Fungus were isolated from Out of the 24 cloacal swabs samples. The isolated organism was bacteria as *E. coli, Salmonella* spp., *Staphylococcus* spp., *Mycoplasma* spp. and fungus were identified. Then out of 20 samples, *E. coli* was 6 (30%), *Salmonella* spp. 5 (25%), *Staphylococci* spp. 4 (20%) and *Mycoplasma* spp. 2 (10%) and at last 3 (15%) fungus grown in subculture media. The frequency distribution of different species of bacterial isolates in different cloacal swabs samples were found in variable condition. So result of the present study indicated that all three types of bacteria, another type of Mycoplasma and other types of fungus were not present in the same cloacal swabs sample which were collected from different aged of poultry, especially in commercial broiler from different located farm in different area that is correlate with the findings of (Shreef *et al.*, 2009; Saleh *et al.*, 2009; Aguirre *et al.*, 1992 and Awad-Alla *et al.*, 2010) with slight variation. The different isolates of *E. coli*, salmonella, and *Staphylococcus* showed identical results in different biochemical tests including Methyl-Red, Voges- Proskauer, and Indole test. The actual causes for which the manifestation of an identical result in biochemical tests by the four groups of known identified isolates were not clear. The manifestation of similar type of biochemical reaction was reported by Ashenafi *et al.*, 1979; Deepti *et al.*, 1982; Ahmed *et al.*, 2004). The colony characteristics of *E. coli* observed in NA, EMB and SS agar were similar to the findings of (Jakaria *et al.*, 2012; Hentschel *et al.*,1979; Muktaruzzaman *et al.*, 2010 and Buxton and Fraser, 1977). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired and motile which was supported by several authors (Freeman, 1979; Buxton and Fraser, 1977 and Merchant and Packer, 1967). The colony characteristics of *Salmonella* spp. observed in NA, SS agar, were similar to the findings of (Sujatha *et al*., 2003; Kleven *et al.*, 1998 and Khan *et al.*, 2005). The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was supported by (Huang *et al.*, 2009 and Hofstad *et al.*, 1972). The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test by (Christensen *et al*., 1992). In accordance with this research Hubalek (2004) also reported a large number of fungal species isolated from poultry.

5. Conclusions

In the context of this study it may be concluded that the microorganism which were collected from cloacal swabs of poultry especially in commercial broiler contains a number of bacteria as like *E. coli, Salmonella* spp. *Staphylococcus* spp. and other type were *Mycoplasma* spp. and also fungus.

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Conflict of interest

None to declare.

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