Occurrences, treatment and antibiotic resistant pattern of colibacillosis and salmonellosis in broiler

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ABSTRACT

An experiment was conducted to investigate present occurrence of colibacillosis and salmonellosis in broiler and to find out the effective antibiotics for treatment of these diseases. The resistant pattern of Escherichia coli and Salmonella sp. against gentamicin, neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin were also observed in Central Disease Investigation Laboratory (CDIL), Bangladesh. The occurrence of colibacillosis was 32.52% and salmonellosis was 13.17% but in mixed infection occurrence was 9.75% with colibacillosis and 2.92% with salmonellosis. Neomycin (38.00%) was mostly used for treatment of colibacillosis and enrofloxacin (25.93%) was used for treatment of salmonellosis. Efficacy of neomycin and enrofloxacin was 92.10% in colibacillosis and 85.81% in salmonellosis respectively. The study also revealed that Escherichia coli were resistant against neomycin (38.88%), oxytetracycline (50.55%), amoxicillin (60.68%), enrofloxacin (50.00%) and ciprofloxacin (30.55%). Salmonella sp. was resistant against neomycin (33.33%), oxytetracycline (63.33%), amoxicillin (63.33%), enrofloxacin (52.38%) and ciprofloxacin (52.38%) respectively. Gentamicin (100%) was highly sensitive for the treatment of colibacillosis and salmonellosis.

Keywords: Salmonellosis, colibacillosis, occurrences, treatments and antibiotic resistant

I. Introduction

Poultry products were one of the most important protein sources for human beings throughout the World. A major portion (44%) of the population of Bangladesh lives below absolute poverty line and the number of landless poor has been increasing by 3.4% per annum (BBS, 2000). About 47.5% people receive less than 1900 calorie per person per day as against the standard 2300 calories. Avian colibacillosis and salmonellosis has been found to be major infectious diseases of all ages of broiler. These diseases occur sporadically or enzootically in most of the countries of the world including Bangladesh. The most important reservoir of *Escherichia coli* was the intestinal tract of animals,

including poultry. In chickens, there were about 109 colony-forming units (CFU) of bacteria per gram of feces. Of these, 106 CFU were *Escherichia coli*, 10-15% of which were pathogenic serogroups and probably infect most mammals and birds thus having a cosmopolitan distribution. Coliforms transmitted between poultry and humans (Ojeniyi, 1989). Egg transmission of pathogenic *Escherichia coli* was common and responsible for high chicken mortality. Pathogenic coliforms were more frequent in the gut of the newly hatched chicks than in eggs from which they hatched (Harry and Hemsly, 1965). Salmonellosis was a serious systemic disease of domestic poultry which cause large scale economic losses (Shivaprasad *et al.* 1997). It causes severe economic losses of the poultry with morbidity and mortality varying in chicken from 10-50% or more (Vaillancourt *et al.*, 1992). Salmonellosis was distributed in many countries of the world, and has economic significance (Barrow *et al.,* 1992). The major of economic loss results from mortality and decrease productivity in affected birds (Otaki, 1995). Antimicrobial resistance, the ability of microorganisms (notably bacteria) to withstand antimicrobial agents (antibiotics), was an important and growing public health issue. However, over the years bacteria that were once controlled by these drugs have developed resistance so that common infections in humans can cause significant harm and even death. *Escherichia coli* was the primary causative agent of cellulitis, septicemia, and air sacculitis in poultry and *Salmonella* sp. was the causative agent of pullorum disease, Fowl typhoid and Fowl paratyphoid (Gomis *et al.*, 1997). Therefore, these were the most significant poultry bacterial pathogen. There were several antimicrobials that have been approved for treatment of *Escherichia coli* and *Salmonella* sp. infections in broiler. However, some of these antimicrobials were not cost-effective, while others were ineffective due to acquired resistance (Bass *et al.*, 1999). The long-term use of antimicrobials for therapy and growth promotion in animals selects for drug resistance in gram-negative pathogens (Levy *et al.*, 1976). Until today very little works have been performed in our country on antibiotic resistant pattern in broiler. Considering all these constraints, this pioneering work has been under taken in this country to determine the present occurrence of colibacillosis and salmonellosis in broiler and find out the most effective antibiotics for treatment of these diseases in Kapasia upazilla of Gazipur district. The resistant pattern of *Escherichia coli* and *Salmonella* sp. against antibiotics (gentamicin, neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin) were also observed in the study.

II. Materials and Methods

The experiment was conducted at Kapasia upazilla of Gazipur district in Bangladesh during 23rd March, 2014 to 14th May, 2014. Antibiotic resistant pattern in broiler were observed in Central Disease Investigation Laboratory (CDIL), Bangladesh. 615 birds from different farms were subjected to post-mortem examination. From them 36 isolates of *Escherichia coli* and 21 isolates of *Salmonella* sp. prepared from single colibacillosis and salmonellosis cases for Cultural Sensitivity (CS) Test. Diagnosis of disease was made on the basis of history, signs and symptoms prior to death of birds, post-mortem lesions of dead birds by post-mortem examination, supported by microbiological examination when indicated, using standard methods (culture at MacConkey agar and SS agar) for bacterial identification. The post-mortem examinations were performed and lesions were recorded as early as possible after death of the birds. Treatment was given according to presumptive diagnosis. Study the type of antibiotics prescribed against those clinical cases. Both *Escherichia coli* and *Salmonella* sp. were gram negative bacteria and were used gram negative bacteria sensitive antibiotics or broad spectrum antibiotics. Gentamicin, neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin were used for the treatment of the colibacillosis and salmonellosis.

Cases suspected for colibacillosis and salmonellosis after post-mortem examination were subjected to microbial examination (Collins and Lyne, 1976). Liver sample was considered for microbial examination. 1 gm of liver samples was taken and kept in the selinite broth. Then tubes of selinite broth with liver were incubated for overnight at 37°C. In case of immediate use after few hours, sample was taken by sterilized cotton swab just after searing. There were many laboratory examinations and techniques for confirm diagnosis of colibacillosis and salmonellosis such as isolation and identification of the organisms, serological test, culturing on various selective media and observation of colony characteristics and also observation of the organisms under microscope after staining. For suspected cases of colibacillosis inoculation from both broth sample and swab sample culturing were done at MacConkey agar and blood agar plate and were kept for overnight incubation

and observed. Both lactose fermenting and non-lactose fermenting colonies were found. More confirmation was made by biochemical tests of pink colored colonies of *Escherichia coli*. Various biochemical tests were done for identification (Cruickshank *et al.,* 1995). Notable performed biochemical tests were SIM (Sulfied Indole Motility Test), TSI (Triple Sugar Iron) agar stab and Urea. Among all confirmatory diagnostic methods culturing on selective media (MacConkey Agar and SS Agar) and observation of colony characteristics as well as Biochemical Test at TSI agar stab were selected for salmonellosis.

After confirmation the isolates as *Escherichia coli* and *Salmonella* sp. were subjected for antibiotic susceptibility tests. Upon *Escherichia coli* and *Salmonella* sp. identification, antibiotic sensitivity test were performed on the isolates by standard technique of EUCAST and recommendation done by the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Antibiotics selected for susceptibility testing included a panel of antimicrobial agents of interest to the poultry industry and human public health authorities. 6 drugs were selected on the basis of their range of activity against enterobacteria and on their use in the poultry farming. The following antibiotics and disc potencies were used: GN: gentamicin (120µg), NEO: neomycin (30µg), CIP: ciprofloxacin (5µg), OT: oxytetracycline (30µg), ENR: enrofloxacin (5µg) and AML: amoxicillin (10µg) from Oxoid Ltd. (Basingstoke, Hampshire, England). The antibiotic susceptibility tests were performed in Muller-Hinton agar (Difco) by EUCAST micro-disc diffusion techniques. Measurement of the growth inhibition zone permitted the classification of each isolates as susceptible, intermediate and resistant according to data collected from Bacteriology Laboratory of CDIL (Central Disease Investigation Laboratory). The result of antibiotic sensitivity test was then recorded and analyzed.

III. Results and Discussion

Total number of diseases

An infectious disease was any disease caused by invasion of a host by a pathogen which subsequently grows and multiplies in the body. Infectious diseases were often contagious, which means they can be spread directly or indirectly from one living thing to another. Diseases (bacterial, viral, fungal etc.) are diagnosed by post-mortem examination includes colibacillosis, salmonellosis, necrotic enteritis, fowl cholera, mycoplasmosis, avian streptococcosis, avian influenza, fowl pox, infectious bursal disease, Newcastle disease, coccidiosis etc.

Table 01. List of diseases available in the study area

Case types	Name of the diseases	No. of birds affected	Frequency (%)
Single Infections	Colibacillosis	200	32.52%
	Salmonellosis	81	13.17%
	Necrotic Enteritis	59	9.59%
	Avian Streptococcosis	5	0.81%
	Mycoplasmosis/CRD	23	3.74%
	Coccidiosis	34	5.53%
	Aspergillosis	15	2.44%
	Infectious Bursal Disease	73	11.87%
	Newcastle disease	37	6.02%
	Fowl pox	4	0.65%
Mixed Infections	Colibacillosis+Avian streptococcosis	3	0.49%
	Colibacillosis+Newcastle disease	11	1.79%
	Colibacillosis+Coccidiosis	6	0.98%
	Colibacillosis+Infectious Bursal Disease	12	1.95%
	Colibacillosis+Mycoplasmosis	10	1.62%
	Colibacillosis+Salmonellosis	18	2.92%
Others	Mycotoxicosis	7	1.14%
	Malnurtition	7	1.14%
	Stress	10	1.62%
	Total	*615*	*100%*

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Some conditions were caused by a disturbance of normal metabolic functions either through inadequate or inappropriate nutrition or impaired nutrient utilization (Jordan and Pattison, 1996). List of different diseases and their frequency in the study area were shown in table 01.

The diagnosed diseases included colibacillosis (32.52%), salmonellosis (13.17%), necrotic enteritis (9.59%), avian streptococcosis (0.81%), mycoplasmosis / CRD (3.74%), coccidiosis (5.53%), aspergillosis (2.44%), infectious bursal disease (11.87%), Newcastle disease (6.02%) and fowl pox (0.65%) as single infections. Mixed infections were available as colibacillosis + avian streptococcosis (0.49%), colibacillosis + Newcastle disease (1.79%), colibacillosis + coccidiosis (0.98%), colibacillosis + infectious bursal disease (1.95%), colibacillosis + mycoplasmosis (1.62%) and colibacillosis + salmonellosis (2.92%). Non-infectious cases were mycotoxicosis (1.14%), malnurtition (1.14%) and stress (1.62%).

Occurrences of colibacillosis and salmonellosis

Among the diseases in commercial broiler farms, the occurrences rate of death due to colibacillosis was the highest. Therefore the occurrences is 32.52% for single colibacillosis case but in case of mixed colibacillosis the occurrences was 9.75% and mixed salmonellosis occurrences was 2.92%. The occurrence of single salmonellosis was 13.17%. Similarly, Bhattacharjee and Majumder (2001) diagnosed the occurrences of avian colibacillosis was highest among the bacterial diseases in chicken at Central Disease Investigation Laboratory (CDIL), Dhaka.

Usage and efficacy of antibiotics for treatment of colibacillosis and salmonellosis

Antibiotics were extremely important tool in the efficient production such as meat and eggs. At sub-therapeutic levels in diets, antibiotics improve growth rate and efficiency of feed utilization, reduce mortality and morbidity and improve reproductive performance. At high levels antibiotics help to prevent disease in exposed animals and to treat diseases (Cromwell, 1999). For the treatment of colibacillosis, gentamicin (18.00%), neomycin (38.00%), oxytetracycline (8.00%), amoxicillin (5.00%), enrofloxacin (15.00%) and ciprofloxacin (16.00%) were used in that area. Gentamicin showed (100%) effective for the treatment of colibacillosis where neomycin was 92.10%, oxytetracycline 62.50%, amoxicillin 50%, enrofloxacin 73.33% and ciprofloxacin 65.62% effective. For the treatment of salmonellosis gentamicin (22.22%), neomycin (17.28%), oxytetracycline (11.11%), amoxicillin (6.17%), enrofloxacin (25.93%) and ciprofloxacin (17.28%) were used in that area. In the treatment of salmonellosis gentamicin showed 100% effective followed neomycin (71.42%), oxytetracycline (55.56%), amoxicillin (60%), enrofloxacin (85.81%) and ciprofloxacin (64.29%).

Comparison of antimicrobial resistant patterns of *Escherichia coli* and *Salmonella* sp.

Antibiotic resistant is a form of drug resistance whereby some sub-populations of a microorganism, usually a bacterial species, were able to survive after exposure to one or more antibiotics. Antibiotic resistant is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century. When bacteria change so antibiotics no longer work in people who need them to treat infections is now a major threat to public health.

Table 02. Sensitivity pattern of *Escherichia coli*

Name of the antibiotics	No. of isolates tested	Sensitivity pattern		
		Resistant (%)	Susceptible (%)	Intermediate (%)
Gentamicin	36	0	100	0
Neomycin	36	38.88	61.12	0
Oxytetracycline	36	50.55	19.45	30
Amoxicillin	36	60.88	11.12	28
Enrofloxacin	36	50	50	0
Ciprofloxacin	36	30.55	69.45	0

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Antibiotics (gentamicin, neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin) were chosen in the study as these antibiotics using in both veterinary and human medical practices. *Escherichia coli* isolates were tested for resistant towards 6 above mentioned antibiotics (Table 2). Resistances were commonly observed against neomycin (38.88%), oxytetracycline (50.55%), amoxicillin (60.88%), enrofloxacin (50%) and ciprofloxacin (30.55%). But high levels of sensitivity were found against gentamicin (100%) and ciprofloxacin (69.45%). Those above-mentioned isolates had considerable sensitivity to neomycin (61.12%). But in previous study all the poultry *Escherichia coli* isolates were found resistant to tetracycline (Bauer *et al.,* 1966). High level of resistance against tetracycline because most of the farmers used commercially available tetracycline in the poultry feed regularly. The farmers used antibiotics largely for three purposes in poultry farms: therapeutic use to treat sick flock; prophylactic use to prevent infections in the flock; as growth promoters to improve feed utilization and production (Barton, 2000). Most of the environmental strains were sensitive to ciprofloxacin (76%), and gentamicin (97%) (Alam *et al.,* 2006). Poultry isolates 72% *Escherichia coli* were found sensitive to gentamicin (Biswas *et al.,* 2001). So, the results of ciprofloxacin resistance and gentamicin sensitivity in poultry isolates were mostly similar with environmental *Escherichia coli*. In another study, a high frequency of resistance to tetracycline, kanamycin, neomycin, cephalothin, streptomycin, and erythromycin was observed. Many strains were resistant to several antibiotics, but no pattern predominates (Allan *et al.,* 1993). Amoxicillin is a broad-spectrum antibiotic of penicillin group. But highest resistance found against these antibiotics 60.88%. No data is still available about the high level of resistance against amoxicillin of *Escherichia coli* isolates in Bangladesh. Enrofloxacin and ciprofloxacin both were belong to 2nd generation fluroquinolones. But variable resistance pattern found *Escherichia coli* isolates against this group and it is highest in enrofloxacin 50%. It is due to more use of enrofloxacin than ciprofloxacin in poultry.

Table 03. Sensitivity pattern of *Salmonella* sp.

Name of the antibiotics	No. of isolates tested	Sensitivity of pattern		
		Resistant (%)	Susceptible (%)	Intermediate (%)
Gentamicin	21	0	100	0
Neomycin	21	33.33	66.67	0
Oxytetracycline	21	63.33	16.67	20
Amoxicillin	21	63.33	16.67	20
Enrofloxacin	21	52.38	47.62	0
Ciprofloxacin	21	52.38	47.62	0

Salmonella sp. isolates were tested for resistant towards 6 above mentioned antibiotics (Table 3). Resistances of *Salmonella* sp. isolates were commonly observed against neomycin (33.33%), oxytetracycline (63.33%), amoxicillin (63.33%), enrofloxacin (52.38%) and ciprofloxacin (52.38%). But high levels of sensitivity were found against gentamicin (100%) and neomycin (66.67%). Similar findings were also reported by Molla *et al.* (2003). Gentamicin is exceptional aminoglycosidic antibiotic which is bacteriocidal for negative bacteria. *Salmonella* sp. isolates were most sensitive to gentamicin among all antibiotics. Highest resistance of *Salmonella* sp. isolates is found 63.33% in case of both oxytetracycline and amoxicillin. Highest resistance found against amoxicillin for both *Escherichia coli* and *Salmonella* sp. isolates is great threat to poultry industry. Intermediate level of resistance found 52.38% in both enrofloxacin and ciprofloxacin of 2nd generation fluroquinolones group. In the present study all *Salmonella* sp. and *Escherichia coli* isolates were susceptible to gentamicin. This may be explained by the limited use and high cost of this antimicrobial that would reduce their frequent utilization both in the veterinary and public health practices in Kapasia area. Poultry farmers avoid gentamicin for being fear of production loss. Further detailed epidemiological and molecular studies are essential on the frequency, sources of acquisition of resistant genes and distribution of antimicrobial resistant of *Escherichia coli* and *Salmonella* sp. in broiler. Antibiotic use should be controlled by the authority to prevent the chance of antibiotic resistance in broiler. Antibiotic resistance monitoring program should also be establish in near future to prevent the global antibiotic resistance problem.

IV. Conclusion

Colibacillosis and salmonellosis found as the potential importance disease for broiler because their occurrences were high in Kapasia upazilla of Gazipur district of Bangladesh. Gentamicin, neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin were commonly used for the treatment of colibacillosis and salmonellosis. Colibacillosis and salmonellosis gradually increase resistant due to indiscriminate use of antibiotics in feed and water. Further detailed epidemiological and molecular studies are essential on the frequency, sources of acquisition of resistant genes and distribution of antimicrobial resistant of *Escherichia coli* and *Salmonella* sp. in broiler. Antibiotic use should be controlled by the authority to prevent the chance of antibiotic resistance in broiler. Antibiotic resistance monitoring program should also be establish in near future to prevent the global antibiotic resistance problem.

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VI. References

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