Bangl. J. Vet. Med. (2004). 2 (2): 99–102

IN VITRO ANTIBIOTIC SENSITIVITY AND THERAPEUTIC EFFICACY OF EXPERIMENTAL SALMONELLOSIS, COLIBACILLOSIS AND PASTEURELLOSIS IN BROILER CHICKENS

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ABSTRACT

Avian salmonellosis (AS), avian colibacillosis (AC) and avian pasteurellosis (AP) have been recognized as important bacterial diseases in poultry associated with morbidity and mortality in Bangladesh. The causative agents of these three diseases were isolated (5 isolates / disease) from dead chickens submitted for diagnosis at the BRAC Poultry Disease Diagnostic Centre, Gazipur during the period from January to December 2002. Five isolates of each of the *Salmonella pullorum*, *Escherichia coli* and *Pasteurella multocida* were evaluated against eight antibiotic containing disc which included ciprofloxacin, gentamicin, ampicillin, chloramphenicol, erythromycin, tetracycline, cephradine and penicillin G. Erythromycin in *S. pullorum* and Ciprofloxacin both in the *E. coli* and *P. multocida* were found highest sensitive, gentamicin, chloramphariсol, cephradine were found moderately sensitive to *S. pullorum*, gentamicin, tetracycline, erythromycin and ampicillin were found moderately sensitive to *E. coli*, and gentamicin ampicillin, cephradine and penicillin G were moderately sensitive to *P. multocida*. Therapeutic trials against experimentally produced *S. pullorum*, *E. coli* and *P. multocida* infection in three groups of broiler chickens showed that cephradine against *S. pullorum* and ciprofloxacin against both in *E. coli* and *P. multocida* were found highly effective both *in vitro* and *in vivo* studies, therefore, cephradine against salmonellosis and ciprofloxacin against colibacillosis and pasteurellosis are effective drugs of choice which could be used to control morbidity and mortality in poultry caused by these diseases.

Key words: Antibiotic sensitivity, therapeutic, salmonellosis, colibacillosis, pasteurellosis, broiler chickens

INTRODUCTION

Avian salmonellosis, avian colibacillosis and avian pasteurellosis are the most important highly fatal bacterial diseases of poultry with worldwide distribution including Bangladesh (Calnek *et al.*, 1997; Samad, 2000). Although there is a published inland report on *in-vitro* sensitivity of *Pasteurella multocida* isolated from chickens and ducks to various chemotherapeutic agents (Choudhury *et al.*, 1985) but there is no published report on *in vitro* antibiotic sensitivity of *Salmonella pullorum* and *Escherichia coli* organisms, causing morbidity and mortality in poultry in Bangladesh (Rahman and Samad, 2003). In addition, there is also no published report on the therapeutic efficacy of antibiotic drugs against experimentally induced avian salmonellosis, avian colibacillosis and avian pasteurellosis in broiler chickens. Therefore, this paper describes the *in vitro* antibiotic drug sensitivity pattern and therapeutic efficacy of certain antibiotics against experimentally induced *S. pullorum*, *E. coli* and *P. multocida* infection in broiler chickens.

MATERIALS AND METHODS

Fifty bacteriological samples suspected for salmonellosis, colibacillosis and pasteurellosis were collected from the BRAC Poultry Disease Diagnostic Centre, Gazipur at necropsy examination in sterilized cotton swabs and put into the sterilized test tube containing nutrient broth. The *Salmonella pullorum, Escherichia coli* and *Pasteurella multocida* organisms were isolated and characterized as described by Rahman *et al.* (2004). These organisms were used for *in vitro* antibiotic sensitivity and experimental infection in broiler chickens for the study of therapeutic trials.

Antibiotic *in vitro* sensitivity test of *S. pullorum* (5 isolates), *E. coli* (5 isolates) and *P. multocida* (5 isolates) was performed with the standardized commercial sensitivity discs (Mast Diagnostics, Mast Group Ltd., Merseyside, UK). Sensitivity to antibiotic was studied mostly on blood agar plates with penicillin G (PG) 10 IU, gentamicin (GM) 10 μg, ampicillin (AP) 10 μg, chloramphenicol (C) 30 μg, tetracycline (T) 30 μg, erythromycin (E) 15 μg, cephradine (CRD) 30 μg and ciprofloxacin (CIP) 5 μg. An amount of 0.5 ml freshly grown pure culture of the bacteria was poured on BA plates and allowed to spread gently over the entire surface with a glass rod spreader. After 1 to 2 minutes the antibacterial discs were placed on the inoculated plates keeping a distance of about 1 cm apart and incubated at 37° C for overnight. The inhibitory effect of the antibacterial to the growth of the culture was recorded.

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The *in vivo* therapeutic efficacy trial was conducted on 12 healthy broiler birds at the age of 52 days. These 12 birds were divided into four equal groups (A to D), each consisting of three birds. Each bird of group A was inoculated with 1.0 ml (5.75 x 10^6 CFU) suspension of *S. pullorum*, group B with 1.0 ml (4.5 x 10^7 CFU) suspension of *E. coli* and group C with 1.0 ml (6.25 x 10^6 CFU) suspension of *P. multocida* orally whereas, birds of group D served as uninfected healthy controls. All these birds were allowed to rear on same feed, water and environmental condition and were observed for clinical signs at every six hours interval. After drug sensitivity test of the re-isolated organisms from cloacal swabs of all the three groups of experimentally infected birds, therapeutic efficacy was conducted in two infected birds of all the three infected groups (A to C) and one infected bird of each of the three groups as untreated infected controls. Each of the two birds of group A was experimentally infected with *S. pullorum* treated with cephradine (Lebac®, Square Pharmaceuticals Ltd.) @ 50 mg / kg body weight at every six hours interval orally for 3 days. Each of the two birds of group B (*E. coli* infected) and group C (*P. multocida*) was treated with ciprofloxacin (Ciprocin® Tab., Square Pharmaceuticals Ltd.) @ 10 mg / kg body weight twice daily orally for 3 days. The therapeutic efficacy was assessed finally on post-mortem examination of all the slaughtered experimental birds.

RESULTS AND DISCUSSION

The results of *in-vitro* antibiotic sensitivity of the isolated *Salmonella pullorum, Escherichia coli* and *Pasteurella multocida* from naturally dead chickens are presented in Table 1 and from isolated organisms from cloacal swabs of experimentally infected broilers are shown in Table 2.

Table 1. Antibiotic sensitivity grade to the different bacteria isolated from the different organs of dead and experimentally infected chickens

S / N	Bacteria isolated	No. of isolate tested	Sensitivity grade	Number (%) of sensitivity to different antibiotics							
				CIP	GM	AP	C	T	E	CRD	PG
1.	*Salmonella pullorum*	5	3^+	0	0	0	0	0	3 (60)	0	0
			2^+	5 (100)	5 (100)	0	5 (100)	0	0	5 (100)	0
			1^+	0	0	5 (100)	0	0	0	0	2 (40)
			R	0	0	0	0	5 (100)	2 (40)	0	3 (60)
2.	*Escherichia coli*	5	3^+	5 (100)	0	0	0	0	0	0	0
			2^+	0	5 (100)	2 (40)	2 (40)	3 (60)	3 (60)	0	0
			1^+	0	0	3 (60)	0	0	2 (40)	0	2 (40)
			R	0	0	0	3 (60)	2 (40)	0	5 (100)	3 (60)
3.	*Pasteurella*	5	3^+	5 (100)	0	0	0	0	0	0	0
			2^+	0	5 (100)	3 (60)	2 (40)	0	0	3 (60)	3 (60)
			1^+	0	0	0	0	0	2 (40)	2 (40)	0
			R	0	0	2 (40)	3 (60)	5 (100)	3 (60)	0	2 (40)

CIP = Ciprofloxacin, GM = Gentamicin, AP = Ampicillin, C = Chloramphenicol, E = Erythromycin, T = Tetracycline, CRD = Cephradine, PG = Penicillin G, 3^+ = Highly sensitive, 2^+ = Moderately sensitive, 1^+ = Sensitive, R = Resistant.

Table 2. Reisolation and identification of different bacteria from the cloacal faeces of experimentally infected chickens and their sensitivity to antibiotics

Sample used	Isolated bacteria (n = 3)	Sensitivity results							
		CIP	GM	AP	C	T	E	CRD	PG
Cloacal swabs	*Salmonella pullorum*	2^+	2^+	1^+	2^+	R	2^+	3^+	R
Cloacal swabs	*Escherichia coli*	3^+	2^+	1^+	R	2^+	2^+	R	R
Cloacal swabs	*Pasteurella multocida*	3^+	2^+	3^+	R	R	R	2^+	2^+

CIP = Ciprofloxacin, GM = Gentamicin, AP = Ampicillin, C = Chloramphenicol, E = Erythromycin, T = Tetracycline, CRD = Cephradine, PG = Penicillin G, 3^+ = Highly sensitive, 2^+ = Moderately sensitive, 1^+ = Sensitive, R = Resistant.

Sensitivity and therapeutic efficacy of bacterial diseases

The *in vitro* drug sensitivity test of bacterial isolates was done for selection of effective therapeutic measures and control. There were slight variations in the sensitivity of antibiotic to the field isolates of bacteria and experimentally isolated bacteria from broiler birds. As for example, field isolates of *Salmonella pullorum* found resistant to erythromycin whereas, experimentally isolated one isolate was highly sensitive to erythromycin and field isolates of *Salmonella pullorum* were found sensitive to penicillin G whereas, experimentally isolated one isolate was found resistant to penicillin G. The field isolates of *E. coli* were found moderately sensitive to chloramphenicol, resistant to tetracycline and less sensitive to erythromycin and penicillin G whereas experimentally isolated *E. coli* isolates were found resistant to chloramphenicol, moderately sensitive to tetracycline and erythromycin, and resistant to penicillin G. The field isolates of *P. multocida were* found moderately sensitive to chloramphenicol, less sensitive to erythromycin and penicillin G whereas, experimentally isolated of *P. multocida* were resistant to chloramphenicol and erythromycin, and moderately sensitive to penicillin G. The variation in the sensitivity of antibiotics of field isolates may be due to outcome of choice and also the indiscriminate use of antibiotic in different disease stage to various birds.

Drug sensitivity pattern of *S. pullorum* showed highly sensitive to erythromycin, moderately sensitive to ciprofloxacin, gentamicin, chloramphenicol, cephradine and less sensitive to ampicillin and resistant to penicillin G. These findings are in agreement with the result of Sharma and Katock (1996) who reported the isolates of *Salmonella typhimurium* sensitive to chloramphenicol, gentamicin and tetracycline; Mitra *et al.* (1997) who reported that the highest number of isolates of *Salmonella* were sensitive to ciprofloxacin and norfloxacin; Dhruba *et al.* (1999) reported that 80% or more of the *Salmonella* isolates were sensitive to gentamicin; chloramphenicol and ciprofloxacin; Jindal *et al.* (1999) reported that majority of the *Salmonella* isolates were sensitive to ciprofloxacin, gentamicin, chloramphenicol and cephalothin; and Shivhare *et al.* (2001) who reported that most of the *Salmonella typhimurium* isolates in birds were sensitive to ciprofloxacin, eurofloxacin and sparfloxacin. The isolates of *Salmonella* sp. were moderately sensitive to gentamicin, pefloxacin, chloramphenicol, doxycycline and ampicillin (Mitra *et al.*, 1997); less sensitive to gentamicin, ampicillin, chloramphenicol and doxycycline (Shivhare *et al.*, 2001). The resistance of *S. pullorum* to penicillin G found in the present study is supported by Sharma and Katock (1996) who reported that *S. typhimurium* was resistant to penicillin, ampicillin and oxytetracycline. The variation in the sensitivity of antibiotic of the isolates of *S. pullorum* from infected chickens may be due to indiscriminate or common use of these antibiotics as feed additives / growth promoters or as preventive and curative purpose as reported by Jindal *et al.* (1999) and Dhruba *et al.* (1999) who found *Salmonella gallinarum* were resistant to 3 to 5 antibiotics.

Ciprofloxacin (100%) was found highly sensitive to all the isolates of *Escherichia coli* followed by gentamicin, tetracycline, and erythromycin were moderately sensitive while ampicillin, penicillin G were less sensitive. This organism was found highly resistant to cephradine (100%) followed by penicillin G (60.0%), chloramphenicol (60.0%) and tetracycline (40.0%). These findings are similar to Prasad *et al.* (1997) who reported highest sensitivity to norfloxacin (80.50%), pefloxacin (77.60%) chloramphenicol (65.60%), ciprofloxacin (53.73%), gentamicin (49.20%) and least sensitive to penicillin and ampicillin. Pandey *et al.* (1998) reported that majority of *E. coli* strains were sensitive to gentamicin, ampicillin and were resistant to tetracycline, benzylpenicillin and chloramphenicol which support the present findings. But there is some variation in the sensitivity tests result with the earlier reports because some antibiotic became resistant due to their commonly used in poultry feed and their disease treatment. Prasad *et al.* (1997) concluded that highest sensitivity to quinolone antibiotics i.e., norfloxacin, pefloxacin and ciprofloxacin because they are recently introduced, have broad spectrum of action and limited use so far, by the poultry farmers which is also suggestive for the present study.

Ciprofloxacin (100%) was found highly sensitive against *Pasteurella multocida*, followed by moderately sensitive to gentamicin (100%), ampicillin (60%), cephradine (60.0%), penicillin G (60.0%), less sensitive to erythromycin (40%) and highly resistant to tetracycline (100%), followed by chloramphenicol (60%), ampicillin (40%), penicillin G (40%); while Prasad *et al.* (1997) showed chloramphenicol (78.9%) was highly effective, followed by pefloxacin (73.6%), gentamicin (73.6%), doxycycline (57.8%), ciprofloxacin (56.1%), cephalexin (40.3%) and penicillin (38.5%). The variation in the sensitivity grade may be due to the indiscriminate use of antibiotics in the poultry industry.

Development of antibiotic resistant strains of bacterial isolates not only pose considerable threat to clinicians and poultry farmers, but is also of public health concern as these resistant strains after ingestion are capable of transferring resistance factor. Thus, judicious use of antibiotics based on their sensitivity pattern should be practiced.

However, it is clear from the drug sensitivity test and therapeutic results that the drug sensitivity may be valuable as background information for future therapy for the effective control of the bacterial diseases, otherwise indiscriminate use of the antibacterial drugs may lead to serious hazards of drug resistance. However, routine laboratory isolation and drug sensitivity test being impracticable, periodical check on the pattern of the drug sensitivity of the organisms becomes all the more significant.

The therapeutic trials against experimentally produced *S. pullorum, E. coli* and *P. multocida* infections were carried out in broilers on the basis of drug sensitivity test and the results are presented in Table 3.

Table 3. Evaluation of the treatment of experimentally infected chickens

S/N	Parameters	*S. pullorum* (n = 3)	*E. coli* (n = 3)	*P. multocida* (n = 3)
1.	Infection	1.0 ml (5.75 x 10^6 CFU) orally	1.0 ml (4.5 x 10^7 CFU) orally	1.0 ml (6.25 x 10^6 CFU) orally
2.	Incubation period	96 hours	96 hours	96 hours
3.	Sub-grouping	Treated (n = 2) Untreated (n = 1)	Treated (n = 2) Untreated (n = 1)	Treated (n = 2) Untreated (n = 1)
4.	Cephradine	All cured Chronic carrier	– –	– –
5.	Ciprofloxacin	– –	All cured Chronic carrier	1 cured Moribund
6.	Gross lesions	– Catarrhal to haemorrhagic enteritis, chalky white materials in the vent, mild congestion of intestine	– Congestion and haemorrhagic streaks in the intestine, air sac cloudy, mild pericarditis and peri-hepatitis	In non-cured one, white nodular granule on liver, pale, and emaciated, hemorrhage on the intestinal mucosa Same as the treated one

Cephradine (Lebac®, Square Pharmaceuticals Ltd.) @ 50 mg / kg body weight at six hours interval orally for 3 days, Ciprofloxacin (Tab Ciprocin®, Square Pharmaceuticals Ltd.) @ 10 mg / kg body weight twice daily orally for 3 days.

The cephradine was found moderately sensitive *in-vitro* against the isolated *S. pullorum* organism which was used @ 50 mg / kg body weight but only at 6 hours interval and resulted disappearance of all the clinical signs at 12 hours of post-treatment and started improvement of the health status of the treated birds. Similarly, ciprofloxacin was found highly sensitive against both the isolated *E. coli* and *P. multocida* organisms, and accordingly, ciprofloxacin @ 10 mg / kg body weight but resulted recovery of all the colibacillosis affected birds, but except one bird, other all pasteurellosis affected chickens were recovered at 12 hours of post-treatment. The non-responsive bird showed lameness, which was not responded due to severely affected conditions.

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