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Short Communication

MONITORING OF LIVER MARKERS IN POULTRY DURING POST INOCULATION OF EXTENDED SPECTRUM β LACTAMASES PRODUCING *ESCHERICHIA COLI* AND AFTER TREATMENT BY CEFTRIAXONE-TAZOBACTAM COMBINATION

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Abstract

Extended spectrum β lactamases (EBSL) producing *Escherichia coli* infections in poultry may adversely affect gut health as well as liver health. *E. coli* infection resistant to antibiotics is sometimes responsible for heavy financial losses to the poultry farmers. Ceftriaxone is a third generation cephalosporin which undergoes significant hepatic clearance in sheep, goat and some other animal species. Diarrhoea was induced experimentally in broiler, Rhode Island Red and Haringhata Black chickens by oral inoculation of 56×10^8 c.f.u. mL^{-1} of ESBL *Escherichia coli* culture and ceftriaxone-tazobactam combination (8:1) was administered at 28.1 mg kg^{-1} intramuscularly twice daily for three days for evaluation of the therapeutic efficacy of the antibiotic combination along with monitoring of liver health during infection and treatment period. Blood samples were collected at 3 days interval up to 21 days post inoculation to study the liver markers like aspartate transaminase, alanine transaminase and alkaline phosphatase. A complete recovery was noticed on 3rd day of treatment which was evidenced by absence of diarrhoea. ESBL *E. coli* infection caused increase in alkaline phosphatase activity during post inoculation and even after treatment indicating intestinal cellular damage. However, aspartate transaminase and alanine transaminase activities did not alter significantly. Therefore, the combination of ceftriaxone and tazobactam in the ratio of 8:1 can be an effective treatment to combat ESBL producing *E. coli* infections resistant to many antibiotics in poultry at 28.1 mg kg^{-1} intramuscularly twice daily for three days.

Key words: Ceftriaxone, ESBL *Escherichia coli*, Liver markers, Poultry, Tazobactam

The poultry sector is growing rapidly in many parts of India which is broadly divided into a highly organized commercial sector (with about 80% of the total market share) and an unorganized sector (with about 20% of the total market share) DADF, 2017. The unorganized sector is also referred to as backyard poultry farming which plays a key role in

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supplementary income generation for the poor people. The Rhode Island Red, an American breed of chicken (*Gallus gallus domesticus*) is considered as backyard dual purpose breed because of its egg laying ability and hardiness. Whereas, Haringhata Black, an indigenous fowl is found in northern part of North 24 Parganas and southern part of Nadia district of West Bengal, India. *Escherichia coli* infections have various disease expressions in domestic birds including salpingitis, synovitis, omphaliitis, diarrhoea and/or chronic respiratory disease. *E. coli* infection may also adversely affect gut health as well as liver health in poultry. Significant morbidity and mortality in the poultry industry occur due to colibacillosis and causes havoc economic losses (Barnes and Gross, 1997). Recommended treatment protocols for *E. coli* infections include antibiotics with a broad spectrum activity and/or sulpha drugs. However, isolates of *E. coli* from poultry are sometimes found to be resistant to common antibacterial drugs.

Extended spectrum β lactamases (ESBLs) are plasmid mediated β lactamases which have the ability to hydrolyze β lactam antibiotics containing an oxyimino group (e.g. ceftazidime, ceftriaxone, cefotaxime or aztreonam). These ESBLs were most commonly found in *Klebsiella pneumoniae*, but are being increasingly found also in *E. coli*, *Proteus mirabilis* and other members of the *Enterobacteriaceae*. The vast majority of ESBLs are derivatives of TEM-1 (the common plasmid mediated β lactamase of organisms such as *E. coli*) or SHV-1 (the common chromosomally mediated β lactamase of *K. pneumoniae*). TEM-1 and SHV-1 can inactivate ampicillin but not the third-generation cephalosporins (Casellas, 1999; Poirel *et al*., 2000). Sometimes, poultry meat is also consumed without proper boiling which may be a source of resistant *E coli* infections in consumers. Hussain *et al.* (2017) reported that prevalence rates of ESBL producing *E. coli* was 46% among broiler chicken meat and was 15% among free range chicken meat. *E. coli* isolated from broiler and free range chicken meat also exhibited 68% and 8% prevalence rates, respectively for multi-drug resistance. A minimum inhibitory concentration (MIC) of ≤0.12 μg mL^{-1} was reported for ceftriaxone against inoculums of 10^5 and 10^6 c.f.u. mL^{-1} of TEM-1 containing *E. coli* (Queenan *et al*., 2004). Tazobactam is an inhibitor of a variety of plasmid-mediated β lactamases which showed no significant differences of overall activity compared to clavulanic acid, another beta lactamase inhibitor (Payne *et al*., 1994). Ceftriaxone-tazobactam combination (3^{rd} generation cephalosporin and β-lactamase inhibitor; 8:1) therapy for ESBL producing *E. coli* infection could be an attractive option. Ceftriaxone was also found to undergo major hepatic clearance in earlier studies in sheep, goat and other animal species (Guerrini *et al.*, 1983; Sar *et al*., 2006, 2008). Therefore, the present research work was undertaken to monitor the liver markers during ESBL *E. coli* infection and following treatment with intramuscular injection of ceftriaxone-tazobactam combination (8:1) at 28.1 mg kg^{-1} twice daily for three days in broiler, Rhode Island Red and Haringhata Black chickens and also to evaluate the efficacy of the antibiotic combination in ESBL producing *E. coli* infection.

A total of eighteen clinically healthy adult chickens (six chickens equally of either sex from each of broiler, Rhode Island Red and Haringhata Black chicken breeds) were collected from the University farm house and quarantined for 14 days. The broilers (Vencobb) were 30 days old but, Rhode Island Red and Haringhata Black chickens were of 12 weeks age. The chickens were caged individually and

commercial finisher and layer feed as well as drinking water was provided *ad libitum*. Pathogenic *E. coli* producing ESBL was isolated from a broiler chicken suffering from diarrhoea (followed by death) in a local poultry farm. The isolated strain belonged to O62 sero-group, pathogenic to experimental chickens and possessed the genes for TEM (bla TEM) which was maintained at Department of Veterinary Microbiology, West Bengal University of Animal & Fishery Sciences, Kolkata. The ESBL producing *E. coli* isolates were also tested for their sensitivity and resistance to ceftriaxone and ceftriaxone-tazobactam combination by disc diffusion method (CLSI, 2003). The ESBL producing *E. coli* isolates showed intermediate sensitivity for ceftriaxone and high sensitivity to ceftriaxone-tazobactam combination (8:1). Therefore, the ceftriaxone-tazobactam combination was preferred over only ceftriaxone for the study as the antibiotic sensitivity test was done *in-vitro* and only ceftriaxone is susceptible to be broken down by ESBLs of *E. coli in vivo*.

For induction of diarrhoea in the experimental broiler (Group 1), Rhode Island Red (Group 2) and Haringhata Black (Group 3) chicken, 56×10^8 c.f.u. mL^{-1} of the bacterial culture was inoculated by oral route (Kumar *et al.*, 2004). However, Haringhata Black chicken did not show any clinical sign following initial challenge of ESBL producing *E. coli* (TEM-1) sub-culture (56×10^8 c.f.u. mL^{-1}) due to their higher resistant to ESBL producing *E. coli* infections (Nandi *et al*., 2016). Due to failure of initial challenge, these chickens were again inoculated orally with a higher second dose (112×10^8 c.f.u. mL^{-1} sub culture) after 21 days of initial oral inoculation of 56×10^8 c.f.u. mL^{-1} sub culture of ESBL producing *E. coli* which resulted in induction of diarrhoea in the Haringhata Black chickens. All the experimental procedures were conducted as per the guidelines of the Institutional Animal Ethics Committee (IAEC), West Bengal University of Animal and Fishery Sciences, dated 09.03.2011.

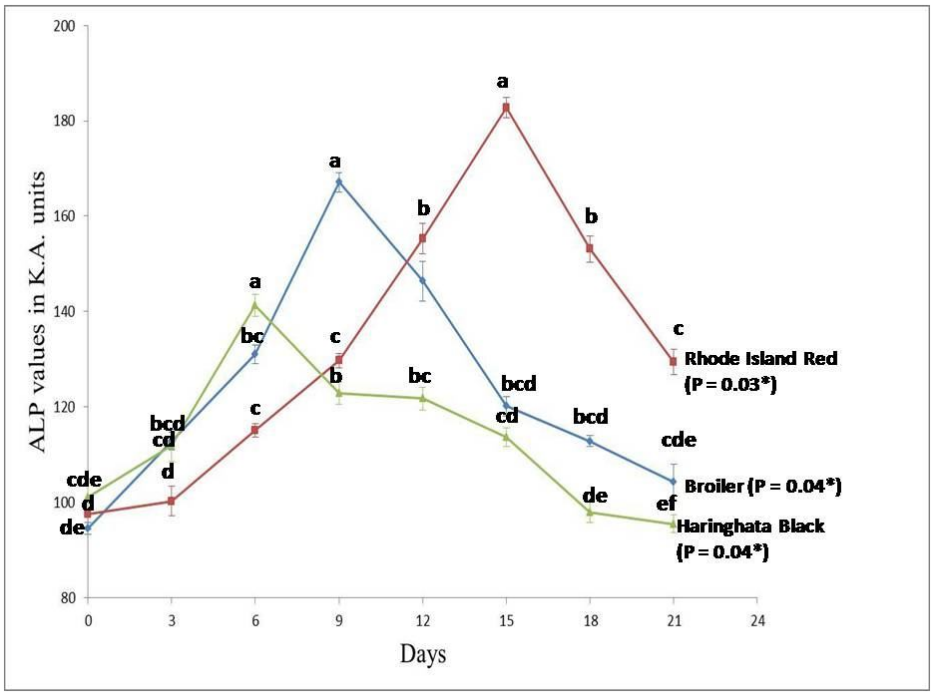
Following induction of diarrhoea ceftriaxone-tazobactam combination (8:1) was administered at 28.1 mg kg^{-1} two times daily (at 12 h interval) intramuscularly for three days. The dosage regimen was calculated on the basis of maintenance of plasma ceftriaxone concentration above MIC level in pharmacokinetic study (under consideration for publication in another journal). Treatment with scheduled dosage regimen of ceftriaxone-tazobactam combination was employed on 7^{th} day post inoculation (after diarrhoea was induced) in broiler and Rhode Island Red chickens and on 8^{th} day post inoculation of second challenge in Haringhata Black chickens. All chickens were closely observed during and after treatment up to a period of 1 month.

Blood samples (1 mL) without anticoagulant were collected from the experimental chickens at 0 day (pre-inoculation) and 3, 6, 9, 12, 15, 18 and 21 days post inoculation of ESBL producing *E. coli.* The collected blood samples were allowed to clot at 25°C and the serum was collected in sterile vials. The serum samples were further centrifuged at 2500 rpm for 15 min to remove residual RBC and it was stored at -20°C. Collected serum samples were utilized for estimation of liver markers like aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities. AST and ALT activity were estimated as per method of Reitman and Frankel (1957), using Span Diagnostic kit (Span Diagnostics Ltd.) and the values were expressed in IU/L. ALP activity was estimated as per method of Kind and King (1954), using Span Diagnostic kit (Span Diagnostics Ltd.) and the value was expressed in King-Armstrong unit (K.A. units). The result data were analyzed statistically using GLM (General Linear Model) method, comparison through LSD (least significant

difference) of IBM SPSS statistic, version 21, 2012.

ALP activity in broiler chickens increased significantly (P<0.05) from 3rd day post inoculation to 21st day post-inoculation compared to pre-inoculation (0 day) value of 94.60±1.19 King-Armstrong (KA) units. Rhode Island Red chickens also showed a significant (P<0.05) increase in ALP activity from 6th day to 21st day post-inoculation of ESBL *E. coli* compared to 0 day value of 97.50±3.07 KA units. Mean ALP values were significantly (P<0.05) higher in Haringhata Black chickens from 3rd day to 15th day post-inoculation of ESBL *E. coli* compared to 0 day value of 101.20±3.80 KA units (Fig. 1). Campbell and Coles (1986) reported that the intestinal isoenzymes make the largest contribution in serum ALP activity in birds. The elevated ALP in chickens indicated intestinal cell damage during the infection caused by ESBL producing *E. coli*.

Broiler chickens showed a significant (P<0.05) increase in AST activity on 3rd day (233.25±1.92 IU/L) post-inoculation of ESBL producing *E. coli* compared to 0 day value of 224.21±1.10 IU/L. However no significant variation of AST activity was observed during rest of the infection period. Rhode Island Red and Haringhata Black chickens did not show significant variation of AST activity during entire infection period (Table 1). Elevated AST values usually indicate liver or muscle damage. Absence of considerable variation in AST values indicated no liver or muscle damage during the infection caused by ESBL producing *E. coli*. However, in contrast to mammals, activity of AST is not liver specific in birds (Lewandowski *et al*., 1986).



[*P<0.05, Means having common alphabet above the line for a specific breed did not differ significantly]

Fig. 1. Mean values of ALP (KA units) in chickens at pre-inoculation time (0 day) and post-inoculation of ESBL producing *E. coli* at 3 days interval up to 21 days

Table 1. Mean values with SE of AST (IU/L) in chickens at pre-inoculation time (0 day) and post-inoculation of ESBL producing *E. coli* at 3 days interval up to 21 days

Chickens	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	P Value
Broiler	224.21^{b} ± 1.10	233.25^{a} ±1.92	228.00^{b} ±1.88	226.33^{b} ±1.39	223.63^{b} ±2.17	229.15^{b} ±1.52	224.42^{b} ±1.89	228.25^{b} ±3.02	0.04*
Rhode Island Red	228.47 ±2.46	228.87 ±0.81	227.35 ±0.73	227.45 ±1.86	232.53 ±2.04	227.93 ±1.95	232.07 ±2.07	229.18 ±1.93	0.08
Haringhata Black	225.31 ±1.28	228.13 ±3.01	223.17 ±1.43	227.00 ±1.77	226.21 ±2.35	227.53 ±3.18	228.42 ±1.79	229.88 ±1.75	0.12

* P < 0.05, Means having common superscript in the same row did not differ significantly

Table 2. Mean values with SE of ALT (IU/L) in chickens at pre-inoculation time (0 day) and post-inoculation of ESBL producing *E. coli* at 3 days interval up to 21 days

Chickens	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	P value
Broiler	23.92^{b} ±0.89	25.47^{b} ±0.95	23.67^{b} ±1.46	26.82^{a} ±1.40	24.93^{b} ±1.02	24.23^{b} ±1.67	26.35^{a} ±1.04	22.14^{b} ±0.81	0.03*
Rhode Island Red	24.90 ±0.76	25.92 ±1.98	25.34 ±1.32	23.32 ±0.75	25.24 ±1.43	26.75 ±0.98	25.25 ±0.81	24.65 ±1.77	0.65
Haringhata Black	24.60 ±1.54	24.43 ±1.84	25.18 ±1.43	25.47 ±0.73	24.47 ±1.18	25.12 ±1.57	24.28 ±1.07	25.16 ±1.16	0.41

* P<0.05, Means having common superscript in the same row did not differ significantly

The ALT activity also increased significantly (P<0.05) on 9th day (26.82±1.40 IU/L) and 18th day (26.35±1.04 IU/L) post inoculation of ESBL producing *E. coli* in broiler chickens compared to 0 day (23.92±0.89 IU/L). But, no significant variation of ALT activity was observed in Rhode Island Red and Haringhata Black chicken during the infection period (Table 2).

ESBL producing *E. coli* infection caused increase in alkaline phosphatase activity in the chickens which may be due to intestinal cell damage. ESBL *E. coli* induced diarrhoea began to subside on 2nd day of treatment with ceftriaxone-tazobactam combination and a complete recovery was noticed on 3rd day of treatment in all the chickens. The treatment was discontinued after 3rd day of treatment due to complete recovery from diarrhoea and no reoccurrence of diarrhoea as well any other clinical signs were noticed during the observation period which correlates with recovery from diarrhoea on 3rd day post treatment. Therefore, ESBL *E. coli* infection as well as the ceftriaxone-tazobactam combination (8:1) administration did not affect liver health of poultry and the ceftriaxone-tazobactam combination (8:1) treatment can effectively combat ESBL producing *E. coli* infection in poultry following administration at 28.1 mg kg^{-1} body weight intramuscularly twice daily for three days.

Conflict of Interest: The authors declare no conflict of interest.

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