Pathogenicity of Coagulase Negative Staphylococcus Chicken Isolates to 10 Days Old Broiler Chickens

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ONE HUNDRED and sixty, 1-day old broiler chicks were grouped into 4 equal groups, at the 10th day birds of groups 1-3 were s.c inoculated with 0.5 ml containing 1.5x10^8 of S.xylis, S. sciuri and S.lentus; respectively and group 4 was noninfected control.

Clinical signs in infected groups started at 2-3dpi as general signs. Signs disappear in Ciprofloxacin treated subgroups 24 hr post treatment and lasted to the 7th day in non treated.

Average body weight gain in S.xylisinfected non treated was the highest (813.90 gm), followed by S. sciuri (778.50gm ) and 773.75 in S. lentus infected treated . FCR was the highest in control (1.69 treated and 1.74 non treated) followed by S. sciuri infected (1.81 non treated and 1.82 treated) and the lowest 1.94 was in S.lentus infected non treated. S. sciuri was reisolated from intestine and spleen (5th dpi) and from intestine (7 and 10 dpi). While S.lentus was reisolated from intestine, liver and spleen (3rd dpi); from intestine and spleen (5th dpi) and intestine (10th dpi).

Histopathological lesion was recorded in infected group as hemorrhages with sinusoidal dilatation, focal areas of vacuolar degeneration, fatty degeneration and shrinkage of hepatocytes in liver, necrotic changes of lymphocytes and vacuolion of corpuscle in spleen. Leucocytic infiltration, degeneration and necrosis of epithelium surface and intraepithelial as well as submucosal leucocytic infiltration were seen in intestine.

In conclusion the injected organisms induce mild subclinical disease with recording of histopathological lesions in liver, spleen and intestine. This area needs more investigation to explore pathogenicity of CoNS in chickens.

Keywords: Broilers, CoNS infection, FCR, Ciprofloxacin, Histopathology

Introduction

Staphylococcosis is a systemic disease of birds caused by avian strains of Staphylococcus spp. [1,2,3]. The infection is common in poultry and game birds; especially in turkeys and broilers. Birds 4 to 6 weeks of age are extremely vulnerable; the most frequent sites of infections are bones, tendon sheaths, and leg joints [4]. Coagulase-negative staphylococci (CoNS) are mostly normal skin commensals and are much less pathogenic than S. aureus [5]. CoNS infections in chickens are considered to be opportunistic [6] and under the appropriate conditions became pathogenic. Research interest in CoNS has increased over the past decade due to their implication in infections in both humans and animals [7,8].

CoNS was reported to be isolated from clinically infected chickens with cellulitis in broiler chickens [9] granulomas in the liver and lungs [10,11], gangrenous dermatitis, sub-dermal abscesses with the wing tips and the dorsal pelvic region [12,13]. S.xylis and S. simulans were recovered from infected bone [14]. Infections with staphylococcus are usually characterized
by increased heterophil counts and marked heterophilic infiltration of tendons, synovial membranes, and other affected organs [15].

Pathogenicity of \( S. \text{ aureus} \) is due to toxic b-hemolysin and plasma coagulase [16]. Cunha Mde et al. [17] analyzed the CoNS virulence factors including hemolysins, lipase, lecithinase, DNAse, thermonuclease, and enterotoxin A, B or C in 37.6% of tested isolates. Zell et al. [18] demonstrate hemolytic activity and the exfoliative toxin A (ETA). Enterotoxins producing \( S. \text{ aureus} \) is the most common cause of food-borne human illness throughout the world [19], the other CoNS species such as \( S. \text{ hyicus}, S. \text{ sciuri}, S. \text{ xylosus} \) or \( S. \text{ cohnii} \) are also important, particularly because of carriage the genes encoding antimicrobial resistance and enterotoxins genes [13,20,21].

The present study is an attempt to detect pathogenicity of CoNS chicken isolates to 10 days old broiler chickens.

**Material and Methods**

**Chickens**

One hundred and sixty , 1- day old broiler chicks were obtained as hatch from commercial hatchery. The used chicks was floor reared and fed on a balanced commercial ration.

**Ration**

The chicks were feed on ration according to the Ross broiler management manual and NRC [22] requirement for broiler. All housed chickens were given ration ad libitum.

**Bacterial strains**

CoNS strains \( S. \text{ xylosis}, S. \text{ sciuri} \) and \( S. \text{ lentus} \) isolated and identified from broiler chicken flocks [23]. The isolates were separately on inoculated 5% blood agar base and Mannitol salt agar (Difco) then incubated for 24-48 hours at 37°C. The resulted colonies were examined for identical morphological character of Staphylococcus species [24].

**Preparation of Bacterial Inoculums**

Used isolates were inoculated into Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours. Infective dose of \( S. \text{ xylosis}, S. \text{ sciuri} \) and \( S. \text{ lentus} \) was adjusted using colony forming units (cfu) was determined by plating tenfold dilutions on Tryptone soya agar as 0.5 ml contains approximately 1.5x10^8 colony forming units (CFU/ml) [25].

**Reisolation of inoculated CoNS**

 Cultures from liver, spleen and intestine of broiler chicks in tryptone soya broth then subcultures on mannitol salt agar finally Gram stained smears from these colonies were examined [24].

**Histopathological examination**

Tissue samples were collected from liver, spleen and intestine of all birds. These samples were fixed in neutral formalin 10% for 48hrs then underwent processing and staining according to Bancroft and Gamble [26]. After fixation, the sample were washed in running water, dehydrated in graduated ethyl alcohol, cleared in xylene and embedded and blocked in paraffin wax. Five microns tissue sections were mounted on clean glass slides and stained with hematoxyline and Eosin (H&E stain).

**Experimental design**

At the 10th day of age the used 160 chicks were divided into 4 equal groups (1-4); 40 chicks each. Chicken of groups 1, 2 and 3 were S/C inoculated with 0.5 ml 1.5x 10^6 of \( S. \text{ xylosis}, S. \text{ sciuri} \) and \( S. \text{ lentus} \); respectively. Birds of group 4 were kept as noninfected control group. At the 15th day with appearance of signs each group was divided into two equal sub groups. Supgroup a was treated with Ciprofloxacilln and subgroup b was kept as infected not treated. The treatment was done using Ciprofloxaix (sensitive in vitro) in a dose of 1cm/litre for 5days .All groups were subjected to daily observation for clinical signs and mortalities with recording of average weekly BWG and FI for calculation of FCR[27].Two birds/ group were randomly weighted and sacrificed at 3, 5, 7 and 10 days post infection (dpi) as well as 2 birds from control group for P.M with recordings of lesions and collection of intestine, spleen and liver samples in formol saline for histopathological examination.

**Results and Discussion**

To study pathogenicity of CoNS in broiler chicks at the 10th day birds of groups 1, 2 and 3); 45 chicks each; were S/C inoculated with 0.5 ml 1.5x 10^6 of \( S. \text{ xylosis}, S. \text{ sciuri} \) and \( S. \text{ lentus} \); respectively. Clinical signs in infected groups at 2-3dpi in the form of general signs of illness including depression , ruffled feather and off food. Group 3 infected with \( S. \text{ lentus} \) showed additionally slight brownish diarrhea in 2 chicks.

After 24 hours post treatment signs started to disappear in treated subgroups while nontreated ones restored their activity at the 10th day (the 5th post treatment ). This result can prove the possible efficacy of used drug as signed in disc sensitivity.

Performance of infected groups those consumed higher feed intake than noninfected control group (Table 1) while the control noninfected showing the lowest feed intake. Average body weight gain of infected non treated subgroups with S. xylosis (Gr 1b) was the highest (813.90 gm), followed by (778.50 gm) in S. sciu (Gr 2b) and 773.75 in the infected treated S. lentus (Gr 3a). In the other hand treatment an infection not markedly affect body weight gain. FCR of control noninfected group was the highest 1.69 in treated and 1.74 in non treated while the lowest rate was in group 3b (1.94) followed by 1.92 in groups 1a and 3a. The result proved no marked effect of CoNS on performance [6]. Becker et al. [28] reported that CoNS have become a major nosocomial pathogen despite the normally sub acute and low inflammatory course of these infections in human.

S. sciuri was reisolated from intestine and spleen of infected chickens at the 5th dpi and from intestine at 7 and 10 dpi. While S. lentus was reisolated from intestine, liver and spleen at the 3rd dpi and from intestine and spleen at the 5th dpi as well as from intestine at the 10th dpi (Table 2). Cheville et al. [29] detected S. hyicus was isolated from birds in all stages of multiple outbreaks of acute severe fibrinopurulent lesions of the eyelids occurred in chickens and turkeys. Awan [30] studied pathogenicity of field isolates in 5-day-old embryonated eggs. S. intermedius or S. lentus demonstrated some pathogenicity in embryos while, these bacterial species caused neither clinical signs of acute or chronic nor mortality in 3-week-old broilers. Osman [31] stated that CNS was one of the main causes of avian cellulitis in chickens.

### Table 1. Average Feed intake/gm, Body weight gain and FCR of CoNS infected Ciprofloxacin treated and nontreated control chickens at 10 days post infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Infection</th>
<th>Treatment</th>
<th>Feed intake/gm</th>
<th>Body weight gain</th>
<th>FCR</th>
</tr>
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<tr>
<td>1</td>
<td>a</td>
<td>S. xylosis</td>
<td>+</td>
<td>1434</td>
<td>748.40</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td></td>
<td>1506</td>
<td>813.90</td>
<td>1.85</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>S. sciuri</td>
<td>+</td>
<td>1330</td>
<td>732.05</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td></td>
<td>1405</td>
<td>778.50</td>
<td>1.81</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>S. lentus</td>
<td>+</td>
<td>1487</td>
<td>773.75</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td></td>
<td>1453</td>
<td>747.40</td>
<td>1.94</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>non infected</td>
<td>+</td>
<td>1213</td>
<td>717.05</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>-</td>
<td></td>
<td>1190</td>
<td>685.25</td>
<td>1.74</td>
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### Table 2. Reisolation of CoNS from infected chicken groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Infection</th>
<th>Subgroup</th>
<th>Treated</th>
<th>DPI</th>
<th>Intestine</th>
<th>Organs</th>
<th>Liver</th>
<th>Spleen</th>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>b</td>
<td>-</td>
<td></td>
<td>3-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>+</td>
<td></td>
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<td>0</td>
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<td>1</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>2</td>
<td>S. sciuri</td>
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<td>-</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<td>1</td>
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<td></td>
<td></td>
<td>a</td>
<td>+</td>
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<td>1</td>
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<td>0</td>
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<tr>
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<td>S. lentus</td>
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<td>-</td>
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<td>2</td>
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<tr>
<td></td>
<td>b</td>
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<td>3-10</td>
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</table>

The recorded histopathological lesion *S. xylosis* infected group 1b: Liver at 3<sup>rd</sup> day dpi showing hemorrhages with sinusoidal dilation (Fig. 1), while focal areas of vacuolar degeneration in the form of fatty change and hydropic degeneration with moderate widening of hepatic sinusoids (Fig. 2) were recorded at 5<sup>th</sup> and 7<sup>th</sup> dpi. Spleen having mild to moderate necrotic changes of lymphocytes especially pyknosis and karyorrhexis (Fig. 3) at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day dpi. Intestine at 3<sup>rd</sup> day dpi showing moderate enteritis having diffuse leucocytic infiltration (mostly macrophages and lymphocytes) with degeneration and necrosis of intestinal mucosa and muscularis mucosa appeared wavy (Fig. 4) as well as focal submucosal mononuclear cell infiltration with mild degenerative changes intestinal epithelium and cystic dilation of a crypt (Fig. 5) at 5<sup>th</sup> and 7<sup>th</sup> dpi.

Fig. 1. Liver showing hemorrhages with sinusoidal dilation. (H & E-stain) (X400).

Fig. 2. Liver showing focal areas of vacuolar degeneration in the form of fatty change and hydropic degeneration with moderate widening of hepatic sinusoids. (H & E-stain) (x200).

Fig. 3. Spleen having mild to moderate necrotic changes of lymphocytes. (H & E-stain) (X400).

Fig. 4. Intestine showing moderate enteritis with degeneration and necrosis of mucosa and muscularis mucosa appeared wavy. (H&E-stain) (X200).

Fig. 5. Intestine showing focal submucosal mononuclear cell infiltration with mild degenerative changes intestinal epithelium and cystic dilation of a crypt. (H & E-stain) (X200)
*S. sciuri* infected group 2b: Liver at 3rd day dpi showing marked widening of hepatic sinusoids with moderate congestion (Fig. 6), at 5th day dpi liver shows mild degeneration with perivascular leucocytic infiltration around central veins (Fig. 7). Moreover, diffuse degenerative changes and congestion of blood vessels and hepatic sinusoids (Fig. 8) were seen at 7th day dpi. Spleen at 5th day dpi showing mild degree of vacuolation of splenic corpuscle (Fig. 9). Intestine at 7th day dpi showing marked enteritis which characterized by severe degeneration and necrosis of intestinal mucosa and leucocytic infiltration (Fig. 10).

Fig. 6. Liver showing marked widening of hepatic sinusoids with moderate congestion. (H & E-stain) (X400).

Fig. 7. Liver show mild degeneration with perivascular leucocytic infiltration around central veins. (H & E-stain) (X200).

Fig. 8. Liver with diffuse degenerative changes and congestion of blood vessels and hepatic sinusoids (H & E-stain) (X200).

Fig. 9. Spleen showing mild degree of vacuolation of splenic corpuscle. (H & E-stain) (X400).

Fig. 10. Intestine showing marked enteritis which characterized by severe degeneration and necrosis of intestinal mucosa and leucocytic infiltration. (H & E-stain) (X200).
S. lentus infected group 3b: Liver at 3rd dpi showing mild degree of vacuolar degeneration in the form of early fatty infiltration and hydric degeneration (Fig. 11), at 5 and 7 days shrinkage of hepatocytes and marked widening of hepatic sinusoids with congestion (Fig. 12) and at 10th dpi of gr 3b showing small fat droplets within hepatocytes with widening and mild congestion of hepatic sinusoids (Fig. 13). Spleen 3rd dpi showing hyperplasia of lymphoid follicle (Fig. 14), vacuolation and necrosis of splenic corpuscle (Fig 9) at 5th day dpi. Intestine shows mild degeneration of surface epithelium with intraepithelial and submucosal leucocytic infiltration (Fig. 15).

Fig. 11. Liver showing mild degree of vacuolar degeneration in the form of early fatty infiltration and hydropic degeneration. (H & E-stain) (X400).

Fig. 12. Liver showing shrinkage of hepatocytes and marked widening of hepatic sinusoids with congestion (H & E-stain) (X200).

Fig. 13. Liver showing small fat droplets within hepatocytes with very mild congestion of hepatic sinusoids (H & E-stain) (X200).

Fig. 14. Spleen showing hyperplasia of lymphoid follicle. (H & E-stain) (X400).

Fig. 15. Intestine of showing mild degeneration of surface epithelium with intraepithelial and submucosal leucocytic infiltration with. (H & E-stain) (X200).

**S.lentus** infected treated gr 3a: Liver at 5dpi showing small fat droplets within hepatocytes with very mild congestion of hepatic sinusoids (Fig. 13) Spleen **S.lentus** infected treated at 5th day dpi showing hyperplasia of lymphoid follicle (Fig. 14).

Stepień-Pyśniak et al. [32] reported that heart revealed multifocal conglomerates of bacterial colonies attached to the valvar endocardium, threads of fibrin, and inflammatory cells with the presence of heterophils. The detection of histological change in infected nontreated groups can support the statement CoNS are shown to have role as opportunistic pathogens [33]. Tsai et al. [34] and Stepień-Pyśniak et al. [32] suggested a strong association between CoNS and endocarditis in broiler chickens. This lesions can be due to action of virulence factors including slime-producing [35,36,37,38], toxic shock syndrome toxin 1 (TSST-1) [18,39], enterotoxin [18,39] and biofilms formation [40].

As Pathogenicity of **S.aureus** is due to toxic b-hemolysin and plasma coagulase [4]. Cunha Mde et al. [17] analyzed the CoNS virulence factors including hemolysins, lipase, lecinthinase, DNAse, thermonuclease, and enterotoxin A, B or C in 37.6% of tested isolates. Zell et al. [18] demonstrate hemolytic activity and the exfoliative toxin A (ETA). Shimaa El-Nagar et al. [21] stated that enterotoxins genes in CoNS see and see were found in 6 isolates with 10.3% for each. Valle et al. [41] found a toxigenic capacity in 45 (16.5%) CoNS isolates, including **S. xylosus**.

In conclusion, from results of this study used organisms can induce subclinical disease while, histopathological lesions in liver, spleen and intestine of infected chickens were recorded. This area needs more investigation to explore factors potentiate pathogenicity of CoNS in infected chickens.

**References**


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