Prevalence of Septic Arthritis Caused by *Staphylococcus aureus* in Poultry Birds at Tandojam, Pakistan

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**Abstract** *Staphylococcus aureus* is known to cause septic arthritis in poultry with significant economic losses. Present study revealed the prevalence of septic arthritis caused by *S. aureus* in broilers and layers in Tandojam, Sindh, Pakistan. One hundred samples from broilers and layers were collected from three sample sites including, hock joint swelling/abscesses, wing/abdominal abscess/injuries and footpad injuries/abscesses. Among 25 samples collected from 1B broilers farm, 69.23% from hock joint swelling/abscesses; 57.15% from wing/abdominal abscess/injuries; and 60.00% from foot pad injuries/abscesses were found positive for *S. aureus*. While, from 25 samples of 2B broilers farm, 73.34% from hock joint swellings/abscesses/injuries; 66.67% from wing/abdominal abscess/injuries; and 75.00% from footpad injuries/abscesses were regarded as positive for *S. aureus* septic arthritis. Similarly from 25 samples of 1L layer farm, 81.25% from hock joint swellings/abscesses/injuries; and 71.43% from wing/abdominal abscesses/injuries were detected as positive for *S. aureus*. Moreover from 2L layers farm, 57.89% from hock joint swellings/abscesses/injuries; and 75.00% from wing/abdominal abscesses/injuries were found positive for *S. aureus*. While, no layer sample of foot pad injuries/abscesses was detected as positive. The study concluded that *S. aureus* is responsible for septic arthritis in majority of both commercial broilers and layers, with higher prevalence in hock joints. Furthermore, layers (64.00%) showed a slightly reduced prevalence of *S. aureus* as compared to broilers (68.00%), with no incidence of causative organism in footpad swellings/injuries.

**Keywords** Septic arthritis, Poultry, *Staphylococcus aureus*, Broiler, Layer

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Among the important diseases of poultry, *Staphylococcus aureus* infection, also called bumble foot, is a common bacterial disease of commercial broilers and layers. It causes significant economic losses through mortality (0-15%) and reduce production performance of birds. *S. aureus* is a normal inhabitant of the skin and upper respiratory tract of diseased and healthy chickens (Shiozawa et al., 1980). It is an important opportunist that can cause superficial to life-threatening illnesses in a variety of animal species. Infection is usually with an incubation period of 2-3 days seen after artificial infection. Toxins are involved in the development of disease and found lethal in birds through necrotizing and hemolytic activities. In poultry, this organism has been implicated in arthritis, osteomyelitis, synovitis, cellulites, dermatitis, endocarditis, septicemia, wound infection, ophthalmitis and omphalitis (Bergmann et al., 1980; Shah et al., 2003; White, 2003).

*S. aureus* is usually the causative agent of septic arthritis, but there is increasing evidence that other species of *Staphylococcus* are also involved. The disease condition vary depending on the bacteria and the host infections have been reported in the bones, joints, tendon sheaths, skin, sternal bursa, navel, yolk sac, liver, lungs, and eyelids (Merck and Corp, 2011).

*Staphylococcal* arthritis is a disease that preferentially affects already-infected joints and causes irreversible joint de-
struction and significant mortality. It is most prevalent in 6 to 12-weeks-old broiler breeders. Septic arthritis is infection of one or more joints that affected by microorganisms. Normally, the joint is lubricated with a small amount of fluid that is referred as synovial fluid or joint fluid. The normal joint fluid is sterile and if removed and cultured in the laboratory no microbes will be found. With septic arthritis, microbes are identifiable in an affected joint fluid. The affect on joints vary somewhat depending on the microbe causing the infection and the predisposing risk factors of the person affected (Huang et al., 2002).

As far as the poultry birds of Sindh province are concerned, no study on the prevalence of septic arthritis caused by the Staphylococcus aureus has been carried out before. The present study is therefore designed to investigate septic arthritis caused by Staphylococcus aureus in commercial broilers and layers in Tandojam and its surroundings.

The present study was carried out to examine the septic arthritis caused by Staphylococcus aureus in poultry birds. For this purpose, two broilers farm in vicinity of Tandojam designated as 1B (Arbor Acre, 03 weeks old) and 2B (Arbor Acre, 05 weeks old); while two layer farms i.e., 1L (White leghorn, 29 weeks old) and 2L (White leghorn, 45 weeks old) were selected for sample collection. A total of one hundred samples fifty each from broilers and layers (25/farm) were collected from three sample sites including, hock joint swellings/abscesses, wing/abdominal abscess/injuries and footpad injuries/abscesses. The samples were collected only from those birds showed the typical signs of septic arthritis like, lameness, swellings, injuries, abscesses and/or oedema at hock joint, foot pad and wings/abdomen (Jordan et al., 2002). The samples were stored in refrigerator at – 4°C and analysed for presence of Staphylococcus aureus.

For the isolation and identification of Staphylococcus aureus, the samples were cultured on different culture media including nutrient, blood and MacConkey’s agar through streaking method and incubated aerobically and anaerobically at 37°C for 24h (Tyagi et al., 2013). Following 24h of incubation, colonies were picked-up by sterilized wire loop and cultured on other media plates. The process of sub-culturing continued until pure growths were obtained. Purity of the isolated organism was determined on the basis of their morphological and cultural characteristics. This was done by making the smear, stained with Gram’s stain and examined under compound microscope. The species of isolated Staphylococcus aureus was confirmed through a series of biochemical and sugar fermentation tests (Kamboh et al., 2007).

All the results were expressed in percentages that were calculated by dividing the number of positive samples with total number of samples x100.

The results regarding the prevalence of septic arthritis in broilers caused by Staphylococcus aureus were presented in Table 1. An overall prevalence of 68.00% was recorded among broilers with 64.00% in 1B and 72.00% in broilers of 2B farm. In 1B farm, out of the 13 hock joint swellings/abcesses/injuries samples, 9 (69.23%) were found positive with Staphylococcus aureus; while from 7 samples of wing/abdominal abscesses/injuries, 4 (57.15%) were detected as positive with Staphylococcus aureus; whereas from 5 footpad injuries/abcesses samples, 3 (60.00%) were recorded as positive. Furthermore, during present investigation from 2B broilers farm, 15 samples from hock joint swellings/abcesses/injuries, 6 from wing/abdominal abscess/injuries and 4 from footpad injuries/abcesses were collected, and among those 11 (73.34%), 4 (66.67%) and 3 (75.00%) were detected positive respectively.

The findings about number and percentage prevalence of septic arthritis in layers caused by Staphylococcus aureus were presented in Table 2. Results showed that both 1L (72.00%) and 2L (56.00%) farms indicated an overall prevalence of 64.00%. Among samples of 1L layer farm; out of 16 hock joint swellings/abcesses/injuries samples, 13 (81.25%) were found positive with Staphylococcus aureus, while from 7 samples of wing/abdominal abscesses/injuries, 5 (71.43%) were detected as positive with Staphylococcus aureus, whereas from 2 footpad injuries/abcesses samples, 0 (00.00%) was recorded as positive. Furthermore, 19 samples of hock joint injuries/abcesses, 4 samples of wing/abdominal abscesses and 2 samples of footpad injuries/abcesses were analyzed from 2L layer farm for presence of Staphylococcus aureus; and 11 (57.89%), 3 (75.00%) and 0 (00.00%) were detected positive respectively.

In present study, we have investigated the prevalence of septic arthritis caused by Staphylococcus aureus in both broilers and layers in Tandojam, Sindh, Pakistan. Presence of Staphylococcus aureus in samples was confirmed by culture and biochemical characterization. The prevalence of Staphylococcus aureus was recorded up to 81%, however some variations (e.g., 1L farm versus 2L farm) were recorded for the prevalence percentage. This might be due to variable management and hygienic conditions and/or excessive stocking density at poultry farm results the diseases outbreaks and onset of chronic conditions (Abel et al., 2014; Kamboh et al., 2009). Gu et al. (2013) reported the Staphylococcus aureus as the most common cause of bacterial arthritis in broiler breeder chickens. They recorded 51.1% mortality in broilers with lesions of swollen joints, gaseous exudates, cartilage injury, and synovial membrane thickening with infiltration of inflammatory cells. Feizi et al. (2012) also investigated the prevalence of Staphylococcus species in broiler breeder flocks in East Azerbaijan province of Iran. They observed 85.71% prevalence of septic arthritis in broilers caused by Staphylococcal species.
Table 1: Prevalence of septic arthritis in broilers of different farms caused by *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Farm identity code</th>
<th>Type of farm</th>
<th>Breed</th>
<th>Age of flock (weeks)</th>
<th>Nature of samples</th>
<th>Site of sample collection</th>
<th>Total No. of samples examined</th>
<th>No. of +ve samples</th>
<th>% of +ve samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>Broilers</td>
<td>Arbor Acre</td>
<td>03</td>
<td>Synovial fluids/pus/blood</td>
<td>Hock joint swellings/abscesses</td>
<td>13</td>
<td>9</td>
<td>69.23 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus and blood</td>
<td>Wing and abdominal injuries/abscesses</td>
<td>7</td>
<td>4</td>
<td>57.15 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus/blood/fluid</td>
<td>Footpad oedema, swellings, injuries</td>
<td>5</td>
<td>3</td>
<td>60.00 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sub-total</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>2B</td>
<td>Broilers</td>
<td>Arbor Acre</td>
<td>05</td>
<td>Synovial fluids/pus/blood</td>
<td>Hock joint swellings/abscesses</td>
<td>15</td>
<td>11</td>
<td>73.34 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus and blood</td>
<td>Wing and abdominal injuries/abscesses</td>
<td>6</td>
<td>4</td>
<td>66.67 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus/blood/fluid</td>
<td>Footpad oedema, swellings, injuries</td>
<td>4</td>
<td>3</td>
<td>75.00 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sub-total</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>50</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of septic arthritis in layers of different farms caused by *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Farm identity code</th>
<th>Types of farms</th>
<th>Breed</th>
<th>Age of flock</th>
<th>Nature of sample</th>
<th>Site of sample</th>
<th>Total No. of sample examined</th>
<th>Number of +ve samples</th>
<th>% of +ve samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1L</td>
<td>Layers</td>
<td>White leghorn</td>
<td>29 weeks</td>
<td>Synovial fluid/pus/blood</td>
<td>Hock joint swellings/abscesses</td>
<td>16</td>
<td>13</td>
<td>81.25 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus and blood</td>
<td>Wing and abdominal injuries/abscesses</td>
<td>7</td>
<td>5</td>
<td>71.43 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus/blood/fluid</td>
<td>Footpad oedema, swellings, injuries</td>
<td>2</td>
<td>0</td>
<td>00.00 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sub-total</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>2L</td>
<td>Layers</td>
<td>White leghorn</td>
<td>45 weeks</td>
<td>Synovial fluid/pus/blood</td>
<td>Hock joint swellings, abscesses/ injuries</td>
<td>19</td>
<td>11</td>
<td>57.89 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus and blood</td>
<td>Wing and abdominal injuries/abscesses</td>
<td>4</td>
<td>3</td>
<td>75.00 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pus/blood/fluid</td>
<td>Footpad oedema, swellings, injuries</td>
<td>2</td>
<td>0</td>
<td>00.00 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sub-total</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>50</td>
<td>32</td>
</tr>
</tbody>
</table>

These results are in agreement with upper limit of our results. Whereas, Rasheed (2011) studied 60 chickens of 30–55 days old with symptoms of arthritis collected from different broiler chicken farms, and detected 50.98% prevalence of septic arthritis caused by *S. aureus*. Furthermore, when 10 chickens were experimentally inoculated by intravenously with 10<sup>7</sup>cfu/ml of *S. aureus* cells, the septic arthritis was developed in 80% chickens.

We recorded the highest prevalence of *S. aureus* in hock joints. This finding is in agreement with the study of Omayma (2005) who examined 84 birds of different breeds and ages obtained from 27 flocks of different localities in Sharkia governorate, and recorded the prevalence of bacterial arthritis caused by the *S. aureus* in 36.6% joints. The author concluded the joints as major site for isolation of *S. aureus*. Daum et al. (2000) reported that poultry birds were susceptible to systemic infections with *S. aureus*. The findings of the present study are in line to the above workers in all respects, they also noted more or less similar results as recorded in this survey regarding septic arthritis in commercial chickens caused by *S. aureus*.

Derivatives of arachidonic acid are known to be the potent mediators for inflammatory diseases like septic arthritis; and cyclooxygenase and 5-lipoxygenase are involved in
metabolisms of arachidonic acid. Furthermore, reactive oxygen species (ROS) and nitric oxide (NO) produced at cellular level are considered as major factors involved in numerous biological processes, like inflammation and immune regulation involved in arthritis and rheumatoid arthritis (Huang et al., 1991; Kamboh and Zhu, 2013). Recent studies have declared that plant origin flavonoids may inhibit the cyclooxygenase and 5-lipooxygenase, thus could modulate the metabolism of arachidonic acid and diminish inflammation. Likewise, the antioxidant effects of flavonoids against ROS and NO production have widely been recognized in recent literature. Hence, these flavonoids could be used as potential antioxidants to modulate the sub-clinical levels of inflammation that act as co-factor for onset of clinical inflammatory diseases like arthritis and/or septic arthritis in farm animals (Guardia et al., 2001; Kamboh et al., 2015).

It is concluded from the study that S. aureus is responsible for septic arthritis in both commercial broilers and layers, however layers showed a slightly lower (about 4%) prevalence of S. aureus infection. A higher prevalence was found in hock joints of chickens with no incidence of causative organism in samples of footpad swellings/injuries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

This work was a part of M. Phil research of first author Nazia. While, Asghar Ali Kamboh, Rahmatullah RindR and Noor Muhammad Soomro acted as supervisor/co-supervisor in this work. However, Kanwar Kumar Malhi, Naem Ullah Durrani, Shakeel Ahmed Lakho and Shabhid Hussain Abro helped in writing and revision of this manuscript.

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