Original Research Article

Incidence of *Staphylococcus aureus* Mastitis in Cows of Faizabad and Sultanpur Districts of Eastern Plain Zone of Uttar Pradesh

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**A B S T R A C T**

*Staphylococcus aureus* is an opportunistic organism and has gain antibiobiotic resistance against several drugs. Cow milk samples (100) were subjected to cultural examination, out of which 68 (68%) samples were found positive for *S. aureus* mastitis. Hemolytic activity on blood agar and biochemical tests viz. catalase test, coagulase test, nitrate test and sugar fermentation tests were performed.

**Keywords**
Cow, mastitis, *Staphylococcus aureus*

**Introduction**

Mastitis (inflammation of mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally (Kumar *et al.*, 2010; AbdEllah, 2013) because of reduced milk production, treatment costs, increased labor, milk with holding following treatment, death and premature culling (Miller *et al.*, 1993; Szweda *et al.*, 2014). Due to multiple etiologies, it always remained a challenge to veterinarian worldwide. Approximately, 140 species of microorganisms have been identified as etiological agents of bovine mastitis. Of these various etiological agents, *Staphylococcus aureus* is a major pathogen associated with bovine clinical and subclinical mastitis (Piepers *et al.*, 2007; Tenhagen *et al.*, 2009; Bhatt *et al.*, 2011; Cervinkova *et al.*, 2013).

The mastitis caused by *S. aureus* is characterized by significantly lower cure rates compared with infections caused by other microorganisms, which may be either as a result of unusually frequent acquisition of antibiotic resistance mechanisms among this group of bacteria or also their ability to form biofilm (slime) (Cramton *et al.*, 1999).

**Materials and Methods**

Milk samples were collected from cows of Sultanpur and Faizabad districts of Eastern Plain Zone of Uttar Pradesh. Initially udders of the cows were examined by visual inspection and palpation for the presence of any lesion, pain, heat and swelling. In addition, milk from each quarter was withdrawn and checked for any change in
colour and consistency. Battery of tests viz California mastitis test and white side tests were employed to test milk for the presence of mastitis.

The affected quarter was washed with tap water, dried properly and the teat was swopped with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was then collected aseptically from a mastitic quarter into sterile bottle after discarding the first 3 to 4 milking streams. Sample from each quarter were transported on ice packs to the bacteriology laboratory and were immediately cultured and stored at 4°C for a maximum of 24 hr until cultured on standard bacteriological media. In this way a total of 100 milk samples showing strong positive reaction were collected for isolation of \textit{Staphylococcus aureus}.

**Bacteriological examination of samples**

The milk samples were subjected to bacteriological examination as per the method prescribed by National committee for clinical laboratory standards (NCCLS, 1997). Isolates of \textit{Staphylococcus aureus} were obtained after subjecting samples to Mannitol salt Agar (MSA) plates followed by nutrient agar slants. The slants were incubated aerobically at 37°C and stored at 4°C until further use. All the samples of \textit{S. aureus} yielded characteristic pink-yellow color colonies on the Mannitol Salt Agar. The isolates obtained as above were subjected to Gram staining. The culture showing violet coloured gram positive cocci, in grape like clusters were primarily considered as of \textit{Staphylococcus aureus}. They were further subjected to hemolytic activity on blood agar and biochemical tests viz. catalase test, coagulase test nitrate test and sugar fermentation tests as per the procedure described by Cappuccino and Sherman (1982).

In Slide coagulase test clumping of cocci within 5 to 10 seconds in the test suspension was taken as positive. In tube coagulase test formation of clot in the test tube indicated positive coagulase test. The method described by Quinn et al. (1994) was followed to determine Catalase test. The production of gas bubbles or effervescence within a few seconds was considered as catalase positive reaction. Development of a distinct red colour which returned to brown rapidly was considered as positive test for Nitrate reduction test. In Sugar fermentation test the sugars used for fermentation reaction were glucose, mannitol and maltose. The observations were made based on colour change and gas production.

**Results and Discussion**

A total of 100 samples were subjected to bacteriological examination out of which 68 samples (68 %) were found positive for \textit{S. aureus}. Among these isolated \textit{S. aureus} isolates from mastitic milk, 62 (91.12%) isolates were tested positive for catalase. A total of 54 (79.41%) isolates were positive in slide coagulase test and 56 (82.35%) isolates were positive in tube coagulase test. Beta hemolytic property was exhibited on sheep blood agar by 50 (73.53%) isolates and nitrate was reduced by 57 (83.82%) \textit{S. aureus} isolates. Methyl reduction (MR) and Voges-Proskauer (VP) test was given by 60 (88.24%) and 58 (85.30%) isolates respectively. In our study 56 (82.35%) isolates of \textit{S. aureus} were found fermenting glucose along with acid production while 46 (67.64%) were gas producer. In Mannitol fermentation 64 (94.12%) isolates were acid producer and 62 (91.18%) were gas producer. In Lactose fermenters, the number of isolates exhibited acid and gas production were 55 (80.88%) and 52 (76.47%) respectively. The details of these tests have been mentioned in table 1 and table 2.
Table 1 Results of Biochemical Test

<table>
<thead>
<tr>
<th>Name of the Biochemical test</th>
<th>Positive S. aureus isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>1. Methyl Red test</td>
<td>60</td>
</tr>
<tr>
<td>2. Voges-Proskaur test</td>
<td>58</td>
</tr>
<tr>
<td>3. Catalase</td>
<td>62</td>
</tr>
<tr>
<td>4. Coagulase</td>
<td></td>
</tr>
<tr>
<td>a. Slide Coagulase</td>
<td>54</td>
</tr>
<tr>
<td>b. Tube Coagulase</td>
<td>56</td>
</tr>
<tr>
<td>5. Nitrate reduction</td>
<td>57</td>
</tr>
<tr>
<td>6. β–haemolysis</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2 Results of sugar fermentation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sugars</th>
<th>Acid Positive Isolates</th>
<th>Gas Producer Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>1.</td>
<td>Glucose</td>
<td>56</td>
<td>82.35</td>
</tr>
<tr>
<td>2.</td>
<td>Mannitol</td>
<td>64</td>
<td>94.12</td>
</tr>
<tr>
<td>3.</td>
<td>Lactose</td>
<td>55</td>
<td>80.88</td>
</tr>
</tbody>
</table>

The high prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intracellularly and localize within micro abscesses in the udder and hence resistant to antibiotic treatment (MacDonald, 1997). The bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. Transmission among cows increases whenever there is lack of effective udder washing and drying, post milking teat dip and drying, inter-cow hand-washing and disinfection, washing clothes and milking machine cups (Radostitis et al., 2007). Therefore, the S. aureus occurrence at a considerable high percentage indicates the alarming situation for dairy sector. Our findings are closely related to the findings of Ankita (2015) who also reported 90.66% Catalase positive Staph aureus in Faizabad district. Ali et al. (2015) also recorded 60% incidence of Staphylococcus aureus mastitis from Bareilly, U.P. Large number of workers from different states of India have also reported Staph aureus to be the main etiological agents of mastitis (Harini et al., 2011; Patel et al., 2012; Sharma et al., 2012; Abhishek Kumar et al., 2013; Awandkar et al., 2013; Mohanty et al., 2013; Charaya et al., 2014; Patnaik et al., 2015).
In recent times, an increasing antimicrobial resistance rate has been recognized in *Staphylococcus aureus* from bovine mastitis (Saini et al., 2012; Wang et al., 2013). Moreover, there is an increased incidence of Methicillin Resistant *S. aureus* (MRSA) all over the world. Further studies are therefore required to study the incidence of methicillin resistant Staphylococcus aureus in this part of eastern plain zone of Uttar Pradesh.

**References**


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