SHORT NOTES

Isolation and antibiogram of coagulase negative Staphylococci from bovine mastitic milk

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Abstract
Milk samples (n=180) collected from Sahiwal and its crossbred cows (n=45) of an organized university dairy farm were confirmed for mastitis and subjected for isolation, identification and antibiotic susceptibility of coagulase negative staphylococci (CoNS). The prevalence of CoNS was found to be 6.1% and antibiogram revealed resistance to most of the routinely used antibiotics. Indiscriminate use of the antibiotics in the farm animal practice coupled with the increasing pathogenicity of the CoNS was suspected to be the issue of major concern.

Keywords: Mastitis, coagulase negative Staphylococci (CoNS), antibiotic resistance.

Introduction
Mastitis (inflammation of mammary gland) is one of the burning problems of economic significance in the dairy industry. The economic loss in India is to the tune of Rs. 6,038.7 million due to sub-clinical and an additional Rs. 2,345.9 million due to clinical mastitis (Singh and Singh, 1994). Bovine mastitis is a complex disease of multifactorial aetiology caused by a variety of microorganism viz. bacteria, fungi, algae, etc of which, mastitis caused by Staphylococci is of paramount clinical significance. It was thought initially that only coagulase positive Staphylococci (CoPS) are associated with pathogenesis leading to disease; however, of late even CoNS have been associated with animal diseases and also emerging as major disease causing agents including mastitis (Matthews et al. 1991). Keeping in view the economic loss caused by the bovine mastitis and emergence of drug resistant CoNS, the present investigation was undertaken with the objective of assessment of prevalence of in CoNS bovine mastitis in an organized dairy farm and to assess the antibiogram of the CoNS isolates so that action plan could be initiated for the effective management of this emerging problem.

Materials and Methods
A total of 180 cow milk samples were collected from cases of clinical mastitis (Sahiwal and its cross-bred cows maintained at organized university dairy farm). Samples were subjected to a battery of tests for confirmation of mastitis using standard protocols (Jorun 1991, Casadevall and Pirofski, 2000). Confirmed mastitic milk samples were further processed for isolation and identification of staphylococci on selective media (Baird-Parker and Staphylococcal Medium SM-110) followed by isolate confirmation based on biochemical reactions (Langlois et al. 1988, Matthews et al. 1988). Confirmed Staphylococcus aureus were subjected to coagulase test and tested against standard Staphylococcus aureus (MTCC) culture as control (Cruickshank et al.
Isolates were subjected to in-vitro antibiotic susceptibility test using 12 different commonly used antibiotics (Bauer et al. 1966).

**Results and discussion**

All the milk samples were confirmed for mastitis based on California mastitis test, white side, pH and physical changes in milk. Mastitic samples showed bacteria viz. Streptococci (28.9%), *E. coli* (22.2%), mixed Streptococcal and Staphylococcal infections (8.9%), Citrobacter (6.7 %) and other gram negative bacteria (8.9 %). About 6.11% samples revealed CoNS. Although CoNS have been predominantly isolated from the bovine milk (Devriese and De Keyser, 1980), they are emerging as pathogens of bovine mastitis leading to considerable loss to the farmers (Timms and Schultz, 1987). Although exact mechanism of the pathogenesis of CoNS in bovine mastitis has not been fully elucidated, yet the role of several toxins and enzymes that contribute to virulence (hemolysin, leucocidin, lipase, proteases, and DNase) has been suggested (Scheifele et al., 1987, Hebert and Hancock, 1985, Watts and Owens, 1987).

Further, increasing resistance to the routinely used antibiotics for CoNS associated with bovine mastitis is emerging as a major concern. The antibiogram of CoNS isolates revealed 100% resistance to erythromycin, amikacin and nitrofurantoin; while other commonly used antibiotics i.e. gentamicin, streptomycin, enrofloxacin, ciprofloxacin, cephalxin, pefloxacin, amoxicillin, oxytetracycline and penicillin-G showed resistance in the increasing order as shown in the Table 1.

Table 1: Antibiogram CoNS isolated from bovine mastitic milk

<table>
<thead>
<tr>
<th>Antibiotic used</th>
<th>Disc content (µg)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>30</td>
<td>27.3</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10</td>
<td>36.4</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>10</td>
<td>36.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30</td>
<td>36.4</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30</td>
<td>54.6</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>5</td>
<td>63.7</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>30</td>
<td>63.7</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>30</td>
<td>72.8</td>
</tr>
<tr>
<td>Penicillin-G</td>
<td>10 units</td>
<td>90.9</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300</td>
<td>100</td>
</tr>
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</table>

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**References**


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