Effect of Infusions of Non-Antibiotic Antibacterials Alone and in Combination with Cephradine on Milk Yield of Buffaloes Affected with Clinical Mastitis

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ABSTRACT

The objective of the present study was to evaluate the effect of four non-antibiotic antibacterials alone or in combination with cephradine in buffaloes on milk yield of mastitis affected quarters. For this purpose, 270 clinically mastitic quarters were grouped in randomized pattern. Non-antibiotic antibacterials viz., 2.5% chlorpromazine (2 ml), 4% lidocaine (10 ml), 10% povidone-iodine (10 ml) and 99.5% dimethylsulphoxide (20 ml) alone and in combination with first generation cephalosporin (cephradine 500 mg) were instilled into clinically mastitic quarters daily for five days. The group administered cephradine alone served as control. Mean milk yield (L/quarter per day) was recorded before administration of treatment and over a period of 4 weeks post initiation of treatment. Among the 4 non-antibiotic antibacterials tested alone, chlorpromazine (CPZ) showed significantly higher (P<0.05) recuperative effect on the milk yield of clinically mastitic quarters of dairy buffaloes. However, dimethylsulphoxide (DMSO) when infused alone, further aggravated (P<0.05) the milk yield loss, indicating negative effect on milk yield improvement. Adjuncting cephradine with each of the non-antibiotic antibacterials, the lidocaine-cephradine group showed the highest effect (p<0.05) on net recovery of milk yield on day 28 post initiation of treatment. It was concluded that that CPZ can be used in clinical mastitis in buffaloes as a low cost alternative to expensive branded antibiotics. Further, the use of lidocaine with cephradnie was superior to all other combination regimens in milk yield recovery.

INTRODUCTION

Mastitis is the most common and economically the most important disease of the dairy industry throughout the world (Sharif and Muhammad 2009). Bubaline mastitis is the disease of milk producing organ of dairy buffalo (Bubalus bubalis) which is also called the ‘black gold’ of South Asia, where 95% of the buffalo milk is produced (Javaid et al., 2009). Buffalo is also recognized as the world second most important milk producing species (McDowell et al., 1995; Bhatti et al., 2009). Mastitis is the single most common reason for antibiotics use in lactating dairy animals (Erskine et al., 2004). However, the poor treatment response of mastitis to antibiotics therapy is a major area of concern for dairy farmers, veterinarians and mastitis researchers. The use of antibiotics for mastitis treatment is attendant with the following important problems: i) Response to antibiotic therapy, in particular in quarters infected with Staphylococcus aureus, is generally very poor, and bacteriological cure rates in clinical S. aureus mastitis vary from 9.7 to 52% (Sole et al., 1994; Sole et al., 2000), ii) Antibacterial therapy of mastitis has been incriminated as a catalyst for developing resistance in pathogenic bacteria both in treated and healthy individuals within a herd (Berghash et al., 1983; Griggs et al., 1994; Teuber, 2001) and iii) The use of antibiotics for mastitis treatment is one of the most important causes of violative antibiotic residues in milk and meat of treated animals (Erskine, 1996).

Thus, there is a pressing need to try some alternative compounds endowed with antibacterial properties to overcome these problems. Recently, a variety of compounds commonly employed in the treatment of pathological conditions of non-infectious etiology have been shown to modify cell permeability and to exhibit
broad-spectrum antimicrobial activity in vitro against bacteria and other micro-organisms (Cederiund and Mardh, 1993; Martin et al., 2008). Such compounds have previously been given the name ‘non-antibiotics’ (Kristiansen and Amaral, 1997). In addition, these compounds have been found to enhance the in vitro activity of certain antibiotics against specific bacteria (Kristiansen, 1990), to render in vitro antibiotic-resistant bacteria susceptible to previously ineffective drugs (Kristiansen et al., 2007; Martins et al., 2008) and to exhibit strong in vitro antimycobacterial activity against clinical strains resistant to one or more conventional antibiotics (Kristiansen, 1990; Williams, 1995; Rodrigues et al., 2008). These compounds, primarily phenothiazines, thioxanthenes and other agents with affinities for cellular transport systems, are characterized by their effects on the plasma membrane of eukaryotic cells (Martin et al. 2008) and have been termed membrane stabilizers (Kristiansen, 1990). Martin, et al. (2008) have reviewed the potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant gram-negative infections.

However, these compounds have not been evaluated in the treatment of mastitis. The objective of the present preliminary study was to determine the effect of infusions of 4 non-antibiotic antibacterials (chlorpromazine, lidocaine, povidone-iodine and dimethylsulphoxide) with and without cephradine (a first generation cephalosporin) on milk yield improvement in mastitic quarters in dairy buffaloes after treatment with these compounds.

**MATERIALS AND METHODS**

A total of 270 clinically mastitic quarters of 249 Nili-Ravi lactating dairy buffaloes (Bubalus bubalis) were selected. Foremilk samples from the affected quarters were collected for bacteriological examination before initiation of treatment and then on day 28 post initiation of treatment (National Mastitis Council, Inc. 1990). Principles of the design of clinical trials with special reference to mastitis therapy, as described by International Dairy Federation (Thorburn, 1990), were followed for selecting trial quarters and their allocation to different treatments and control groups by randomization. Animals previously treated for mastitis during the current lactation were not included in the panel of experimental subjects. Similarly, only those quarters were selected which had contralateral normal quarters.

Animals selected were from buffaloes managed at the Livestock Experimental Station, University of Agriculture, Faisalabad and six private buffalo dairy farms. All experimental buffaloes were managed in tie-stall and loose housing system during the experimental period. These buffaloes received a diet of concentrate mixture and green fodder. In a cut-and-carry feeding system, chopped green fodder plus chaffed wheat straw were fed. Standard mastitis control practices (e.g. post milking antiseptic teat dipping, dry period antibiotic therapy, segregation or culling of mastitic animals) were not in practice at any of the farms.

The milk yield of the quarters affected with mastitis was subtracted from the milk yield of contralateral normal quarters before infusion of non-antibiotic antibacterials into mastitic quarters. Similarly, the milk yield of mastitis affected quarters treated with non-antibiotics was subtracted from the milk yield of opposite normal quarter. Usually, treated quarters do not regain their milk production in a week or two. So, a period of 28 days after the commencement of treatment was adopted for milk yield record to compare the mean yield of different groups.

The present study involved two experiments. The experiment 1 aimed at evaluation of non-antibiotic antibacterials alone in the treatment of bubaline clinical mastitis. For this purpose, 30 mastitic quarters were treated by intramammary route with each of the 4 non-antibiotic antibacterials (Table 1), using human intravenous catheter no. 22 (Vasocan Braunule™) attached to 50 ml plastic syringe. Only 2-3 mm anterior tip of the catheter was introduced into the teat (partial insertion) for infusion.

Immediately before treatment, mean milk yields of the mastitis quarters as well as opposite normal quarters were recorded. Similarly, at day 28 post initiation of treatment, mean milk yield of treated quarters and contralateral normal quarters were recorded.

**Table 1: Evaluation of non-antibiotic antibacterials alone in the treatment of bubaline clinical mastitis**

<table>
<thead>
<tr>
<th>Non-antibiotic antibacterials</th>
<th>Product and manufacturer</th>
<th>Volume and amount infused into each quarter per day</th>
<th>No. of quarters infused</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine HCl (CPZ)</td>
<td>Inj. Largactil™, Aventis Pharma, Pakistan</td>
<td>2 ml (50mg) + 38 ml normal saline</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Lidocaine HCl (Lid)</td>
<td>Xylocaine™ (4%), Barrett Hodgson, Pakistan</td>
<td>10 ml + 30 ml normal saline</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Povidone-iodine (PI)</td>
<td>Pyodine™ solution (10%), Brookes Pharmaceutical Lab., Pakistan</td>
<td>10 ml + 30 ml normal saline</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Dimethylsulphoxide (DMSO)</td>
<td>Dimethylsulphoxide (99.5%) Sigma-Aldrich, GhbH, Germany</td>
<td>20 ml + 20 ml normal saline</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Cephradine (control) (Ceph)</td>
<td>Inj. Velosef, Bristol-Mayer Squibb, Pakistan</td>
<td>500 mg + 40 ml normal saline</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>
Experiment II involved the evaluation of non-antibiotic antibacterials in combination with antibiotic (cephradine) in the treatment of bubaline clinical mastitis. All 4 non-antibiotic antibacterials (chlorpromazine, lidocaine, povidone-iodine and dimethylsulphoxide) were evaluated in regimens similar to those in experiment I, except that 500 mg cephradine (Inj. Velosef™, Bristol-Myers Squibb, Pakistan) was added to daily infusions of each non-antibiotics antibacterial in 30 mastitis quarter in each group. Mean milk yield loss of the affected quarters was analyzed by analysis of variance to compare means of each group. Mean milk yield loss of the affected quarters in losses of milk yield was observed in CPZ group (15.38), followed by Lid (7.53) and PI (5.68%) group. Contrarily, in DMSO treated group, the quarters treated with DMSO registered a further decline. In other words, a significant (P<0.05) reduction in net milk yield loss in quarters treated with povidone-iodine was very poor (5.68%), which might have been due to its irritability on udder tissue. Udder irritation produced by antimastitic preparations is one of the main criteria in the evaluation experiments (Muhammad et al., 1990). More irritant the drug, the lesser it is desirable for intramammary infusion (Uvrov, 1971).

Thus, the highest percent net recovery of milk yield was observed in CPZ group (15.38), followed by Lid (7.53) and PI (5.68) group. Contrarily, in DMSO treated group, the milk yield loss increased further instead of decline. In other words, a significant (P<0.05) reduction (Table 3) in losses of milk yield was observed in CPZ group as compared to other treatment groups. This may be due to strong antibacterial effect of CPZ (Williams, 1995; Amaral et al., 1996; Martins et al., 2008). On the other hand, the quarters treated with DMSO registered a further significant (P<0.05) increase in loss of milk yield. This may be attributed to inability of dimethylsulphoxide to control bacterial infections, as this chemical possesses only a weak antibacterial activity (Plumb, 1999). The net recovery of milk yield in quarters treated with povidone-iodine was very poor (5.68%), which might have been due to its irritability on udder tissue. Udder irritation produced by antimastitic preparations is one of the main criteria in the evaluation experiments (Muhammad et al., 1990). More irritant the drug, the lesser it is desirable for intramammary infusion (Uvrov, 1971).

In the present study, although all four non-antibiotic antibacterials with and without cephradine were administered by intramammary infusion, no attempt was made to study their comparative irritability in terms of increases in milk somatic cell counts. This is one of the shortcomings of the present study which should be addressed in any similar future investigation on the use of non-antibiotic antibacterials in mastitis treatment. Milk somatic cell count has been shown to increase due to mastitis (Khan and Khan, 2006; Sharif et al., 2007).

Considering combination regimens (Table 2), the pre-treatment milk yield losses in quarters treated with CPZ, Lid, PI and DMSO in combination with cephradine were 29.10, 34.75, 31.42 and 33.10%, respectively. The corresponding values on day 28 post-initiation of treatment were 11.85, 13.47, 19.01 and 15.38%, respectively. The shortest period of recovery of milk yield was observed in CPZ group (15.38), followed by Lid (7.53) and PI (5.68%) group. Contrarily, in DMSO treated group, the quarters treated with DMSO registered a further decline. In other words, a significant (P<0.05) reduction (Table 3) in losses of milk yield was observed in CPZ group as compared to other treatment groups. This may be due to strong antibacterial effect of CPZ (Williams, 1995; Amaral et al., 1996; Martins et al., 2008). On the other hand, the quarters treated with DMSO registered a further significant (P<0.05) increase in loss of milk yield. This may be attributed to inability of dimethylsulphoxide to control bacterial infections, as this chemical possesses only a weak antibacterial activity (Plumb, 1999). The net recovery of milk yield in quarters treated with povidone-iodine was very poor (5.68%), which might have been due to its irritability on udder tissue. Udder irritation produced by antimastitic preparations is one of the main criteria in the evaluation experiments (Muhammad et al., 1990). More irritant the drug, the lesser it is desirable for intramammary infusion (Uvrov, 1971).

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<table>
<thead>
<tr>
<th>Non-antibiotic antibacterials alone and in combination with cephradine</th>
<th>Milk yield of mastitic quarters before and after treatment</th>
<th>Milk yield of contralateral normal quarters</th>
<th>Difference of mean</th>
<th>Reduction in milk yield loss of affected quarters (%)</th>
<th>Net recovery of milk yield of treated quarters on day 28 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPZ</td>
<td>A 0.97 ± 0.10</td>
<td>1.42 ± 0.11</td>
<td>0.45 ± 0.05</td>
<td>31.69</td>
<td>15.38</td>
</tr>
<tr>
<td></td>
<td>B 1.18 ± 0.13</td>
<td>1.41 ± 0.11</td>
<td>0.23 ± 0.07</td>
<td>16.31</td>
<td></td>
</tr>
<tr>
<td>Lid</td>
<td>A 1.0 ± 0.09</td>
<td>1.43 ± 0.11</td>
<td>0.43 ± 0.12</td>
<td>30.06</td>
<td>7.53</td>
</tr>
<tr>
<td></td>
<td>B 1.10 ± 0.07</td>
<td>1.42 ± 0.09</td>
<td>0.32 ± 0.02</td>
<td>22.53</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>A 0.89 ± 0.11</td>
<td>1.33 ± 0.12</td>
<td>0.44 ± 0.08</td>
<td>33.08</td>
<td>5.68</td>
</tr>
<tr>
<td></td>
<td>B 0.98 ± 0.17</td>
<td>1.35 ± 0.11</td>
<td>0.37 ± 0.11</td>
<td>27.4</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>A 1.03 ± 0.07</td>
<td>1.37 ± 0.07</td>
<td>0.34 ± 0.09</td>
<td>24.8</td>
<td>-9.75</td>
</tr>
<tr>
<td></td>
<td>B 0.89 ± 0.08</td>
<td>1.36 ± 0.07</td>
<td>0.47 ± 0.08</td>
<td>34.55</td>
<td></td>
</tr>
<tr>
<td>Ceph (control)</td>
<td>A 0.95 ± 0.09</td>
<td>1.45 ± 0.09</td>
<td>0.50 ± 0.08</td>
<td>34.48</td>
<td>13.94</td>
</tr>
<tr>
<td></td>
<td>B 1.16 ± 0.13</td>
<td>1.46 ± 0.08</td>
<td>0.30 ± 0.09</td>
<td>20.54</td>
<td></td>
</tr>
<tr>
<td>CPZ + Ceph</td>
<td>A 0.95 ± 0.12</td>
<td>1.34 ± 0.10</td>
<td>0.39 ± 0.06</td>
<td>29.10</td>
<td>17.25</td>
</tr>
<tr>
<td></td>
<td>B 1.19 ± 0.11</td>
<td>1.35 ± 0.10</td>
<td>0.16 ± 0.09</td>
<td>11.85</td>
<td></td>
</tr>
<tr>
<td>Lid + Ceph</td>
<td>A 0.92 ± 0.11</td>
<td>1.41 ± 0.10</td>
<td>0.49 ± 0.10</td>
<td>34.75</td>
<td>21.28</td>
</tr>
<tr>
<td></td>
<td>B 1.22 ± 0.14</td>
<td>1.41 ± 0.10</td>
<td>0.19 ± 0.08</td>
<td>13.47</td>
<td></td>
</tr>
<tr>
<td>PI + Ceph</td>
<td>A 0.96 ± 0.26</td>
<td>1.40 ± 0.11</td>
<td>0.44 ± 0.07</td>
<td>31.42</td>
<td>12.41</td>
</tr>
<tr>
<td></td>
<td>B 1.15 ± 0.11</td>
<td>1.42 ± 0.11</td>
<td>0.27 ± 0.07</td>
<td>19.01</td>
<td></td>
</tr>
<tr>
<td>DMSO + Ceph</td>
<td>A 0.97 ± 0.19</td>
<td>1.45 ± 0.10</td>
<td>0.48 ± 0.10</td>
<td>33.10</td>
<td>17.72</td>
</tr>
<tr>
<td></td>
<td>B 1.21 ± 0.14</td>
<td>1.43 ± 0.09</td>
<td>0.22 ± 0.12</td>
<td>15.38</td>
<td></td>
</tr>
</tbody>
</table>

CPZ = Chlorpromazine, Lid = Lidocaine, DMSO = Dimethylsulphoxide PI = Povidone – iodine, Ceph = Cephradine
A = Pre-treatment; B = Post-treatment
Means sharing different letters in a column or row are significantly different (P<0.05).

Thus, the highest net recovery of milk yield on day 28 post-initiation of treatment was 21.28% for Lid + Ceph group, followed DMSO + Ceph, CPZ + Ceph and PI + Ceph groups. Among the quarters receiving combination treatments, higher reduction (P<0.05) in the loss of milk yield was observed in Lid + Ceph, group (Table 3) compared to other treatment groups. This may be due to strong antibacterial effect of lidocaine against Gram negative bacteria and that of cephradine against Gram positive bacteria (Schmidt and Rosenkranz, 1970).

In summary, among the 4 non-antibiotic antibacterials tested alone, chlorpromazine showed a relatively more promising recuperative effect on the milk yield of clinically mastitic quarters of dairy buffaloes. Dimethylsulphoxide, when infused alone, aggravated the milk loss of clinically mastitic quarters. Adjuncting cephradine with each of the 4 non-antibiotic antibacterials showed that the lidocaine-cephradine combination had the highest effect (P<0.05) on the net recovery of milk yield loss at day 28 post initiation of treatment.

The present study was the first one on the use of chlorpromazine and lidocaine in the treatment of mastitis. In view of a small number of quarters (n=30) on which each of the 4 non-antibiotic antibacterials were tested, a larger field trials involving a larger number of mastitis affected animals and quarters is clearly warranted.

**REFERENCES**


References


