MICROBIOLOGICAL QUALITY OF RAW MILK AND ITS PUBLIC HEALTH SIGNIFICANCE

Chandra Shekhar, E. Motina and Sunil Kumar
College of Veterinary Science and Animal Husbandry,
Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad-224 229, India.

ABSTRACT
A total of 60 milk samples collected from different milk vendors of different areas of the Faizabad district were subjected to different microbial counts and antibiotic sensitivity of bacterial isolates. Range (Mean ± SE) of different microbial counts viz., coliform, E. coli, faecal streptococci, *Staphylococcus aureus*, yeast and mould, and total viable counts were found as 0.00 - 4.50 (3.00 ± 1.15), 0.00 - 1.20 (0.70 ± 0.46), 1.00 - 2.80 (1.80 ± 0.64), 0.00 - 3.00 (2.10 ± 1.14), 0.00 - 2.40 (1.00 ± 0.78), and 3.80 - 7.20 (5.50 ± 0.99) log_{10} cfu/ml, respectively. On the basis of different microbial counts viz., coliform, *E. coli*, faecal streptococci, *Staphylococcus aureus*, yeast and mould, and total viable count, the milk samples of rejected category were found as 58.33%, 50%, 51.67%, 56.67%, 41.60%, and 53.33%, respectively. *E. coli; Salmonella* spp.; *Klebsiella pneumoniae; Proteus* ammonia*; Campylobacter jejuni; Staphylococcus aureus* and *Bacillus cereus* isolated in this study showed highest sensitivity against norfloxacin and ofloxacin (93.33% each); amikacin, ciprofloxacin, gentamicin and chloramphenicol (100% each); ofloxacin (100%); gentamicin (100%); ofloxacin (75%); enrofloxacin (91.43%) and amikacin (100%), respectively. Moreover, some isolates of *E. coli, Salmonella* spp. and *Campylobacter jejuni* showed multiple resistance against some antibiotics.

Key words : Milk, Microbial counts, Bacterial isolates, Antibiotic sensitivity.

INTRODUCTION
Milk besides being a rich source of nutrients also supports the multiplication of microorganisms such as *Salmonella, E. coli, Listeria, Campylobacter, Staphylococcus aureus, Bacillus cereus, Yersinia* etc. Not only the presence of pathogenic microorganisms and their toxic metabolites but also other spoilage microbial species causes adverse effect on food quality and safety during primary production, collection, transportation and distribution of milk (Rajorhia, 2006). Raw, unprocessed and unpackaged milk sold by milk vendors is quite below standards in hygienic point of view. However, little information is available on microbiological quality of raw milk sold in the Faizabad district. Therefore, a study was planned to assess the microbiological quality of milk sold by the milk vendors of different areas of this district.

MATERIAL AND METHODS
A total of 60 raw milk samples were collected aseptically in the sterile bottles from different milk vendors of different areas of the Faizabad district of Uttar Pradesh, India and brought to the laboratory under chilled condition. All milk samples were subjected to different microbial counts viz., coliform, *E. coli*, faecal streptococci, *Staphylococcus aureus*, yeast and mould, and total viable count as per the standard methods of APHA (1984). For conducting different microbial counts viz., coliform, *E. coli*, faecal streptococci, *Staphylococcus aureus*, yeast and mould, and total viable count the media used were, violet red lactose bile (VRLB) agar, eosin methylene blue (EMB) agar, Slanetz and Bartley agar, mannitol salt agar, yeast and mould count agar, and milk plate count agar, respectively. The data obtained from different microbial counts were presented as log_{10}.
cfu/ml and processed for statistical analysis as per the standard methods of Snedecor and Cochran (1994). Milk samples were categorized into two groups that is samples within the permissible limits of microbial counts and samples beyond the permissible limits of microbial counts. The standard plate count and coliform counts of raw milk are \(5 \times 10^6\) and \(1 \times 10^2\), respectively as per the standards described by Yadav et al. (1993).

All milk samples were subjected to isolation and identification of pathogenic bacteria on the basis of cultural characters, morphological characters and biochemical reactions as per the standard methods described by Collee et al. (1996). The biochemical tests used for identification of different microorganisms were indole, methyl red and Voges-Praskauer test; citrate utilization test; glucose, lactose and sorbitol fermentation tests; urease and phenylalanine deaminase test; growth in KCN medium; oxidase and coagulase tests; nitrate production test and \(H_2S\) production on TSI medium. For isolation of Salmonella, buffered peptone water was used as pre-enrichment medium, selenite broth as a selective enrichment medium and brilliant green agar as a selective medium. For isolation of Campylobacter, milk samples were enriched in an enrichment broth (Preston Campylobacter enrichment broth) and incubated at \(42^\circ C\) for 24 hours under microaerophilic condition (5\% \(O_2\), 10\% \(CO_2\) and 85\% \(N_2\)). After incubation the enriched broth cultures were subcultured on to plates of selective medium (Campylobacter blood free medium) and incubated at \(42^\circ C\) for 48 hours under microaerophilic condition. Growth of watery colonies on the plates was subjected to Gram's staining and biochemical test for identification of organisms.

Isolated and identified bacteria were also subjected to antibiotic sensitivity by using disc diffusion technique of Bauer et al. (1966). Antibiotic discs (Hi-media) used were amikacin (10mcg), amoxycillin (10mcg), norfloxacin (10mcg), ciprofloxacin (10mcg), enrofloxacin (5mcg), ofloxacin (2mcg), gentamicin (10mcg), erythromycin (15mcg), tetracycline (30mcg) and chloramphenicol (30mcg). The zone of inhibition around the colonies were measured and compared as per manufacturer’s instructions to interpret the results as sensitive or resistant.

**RESULTS AND DISCUSSION**

The analysis of milk samples revealed the range (Mean SE) of different microbial counts viz., coliform, *E. coli*, faecal streptococci, *Staphylococcus aureus*, yeast and mould, and total viable count as 0.00 - 4.50 (3.00 ± 1.15), 0.00 - 1.20 (0.70 ± 0.46), 1.00 - 2.80 (1.80 ± 0.64), 0.00 - 3.00 (2.10 ± 1.14), 0.00 - 2.40 (1.00 ± 0.78), and 3.80 - 7.20 (5.50 ± 0.99) \(\log_{10}\) cfu/ml, respectively (Table 1). Almost similar total viable count, coliform count and *E. coli* count were recorded as 5.70 ± 0.13, 3.10 ± 0.17 and 1.01 ± 0.29, respectively by Prejit et al. (2006). However, comparatively higher faecal streptococci count; and yeast and mould count (\(\log_{10}\) cfu/ml) as 2.57 ± 0.12; and 1.84 ± 0.24 were recorded by Prejit et al. (2006).

On the basis of different microbial counts viz., coliform, *E. coli*, faecal streptococci, *Staphylococcus aureus*, yeast and mould count, and total viable count

<table>
<thead>
<tr>
<th>Microbial counts</th>
<th>Range (log_{10} cfu/ml)</th>
<th>Mean (log_{10} cfu/ml)</th>
<th>Number of samples within the permissible limit (%)</th>
<th>Number of samples beyond the permissible limit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total viable count</td>
<td>3.80 - 7.20</td>
<td>5.50 ± 0.99</td>
<td>28 (46.67)</td>
<td>32 (53.33)</td>
</tr>
<tr>
<td>Coliform</td>
<td>0.00 - 4.50</td>
<td>3.00 ± 1.15</td>
<td>25 (41.67)</td>
<td>35 (58.33)</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.00 - 1.20</td>
<td>0.70 ± 0.46</td>
<td>30 (50.00)</td>
<td>30 (50.00)</td>
</tr>
<tr>
<td>Faecal streptococci</td>
<td>1.00 - 2.80</td>
<td>1.80 ± 0.64</td>
<td>29 (48.33)</td>
<td>31 (51.67)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.00 - 3.00</td>
<td>2.10 ± 1.14</td>
<td>26 (43.33)</td>
<td>34 (56.67)</td>
</tr>
<tr>
<td>Yeast and moulds</td>
<td>0.00 - 2.40</td>
<td>1.00 ± 0.78</td>
<td>35 (58.33)</td>
<td>25 (41.67)</td>
</tr>
</tbody>
</table>
58.33%, 50%, 51.67%, 56.67%, 41.67%, and 53.33% samples, respectively; were judged to be of rejected category as per the standards described by Yadav et al. (1993). Almost similar findings of rejected grade of milk samples of 57.50%, 64.20% and 40.80%, on the basis of standard plate count, coliform count, and staphylococci count, respectively, have also been reported by Kumar et al. (2004).

Pathogenic bacteria viz., E. coli, Salmonella spp., Klebsiella pneumoniae, Proteus ammoniae, Campylobacter jejuni, Staphylococcus aureus and Bacillus cereus were recovered in our study have also been reported in milk by earlier researchers (Kumari and Kalimuddin, 2003; Kumar et al. 2004; Hussain et al. 2005).

A total of 98 bacterial isolates comprised E. coli (30), Salmonella spp. (2), Klebsiella pneumoniae (5), Proteus ammoniae (12), Campylobacter jejuni (4), Staphylococcus aureus (35) and Bacillus cereus (10) were recovered from 60 milk samples. The most prevalent bacterium in the milk samples was Staphylococcus aureus, which might be due to the production of milk under unhygienic conditions and unclean environment.

The results of antibiotic sensitivity pattern of E. coli, Salmonella spp., Klebsiella pneumoniae, Proteus ammoniae, Campylobacter jejuni, Staphylococcus aureus and Bacillus cereus showed highest sensitivity against norfloxacin and ofloxacin (93.33% each), amikacin (100%), ofloxacin (100%), gentamicin (100%), ofloxacin (75%), enrofloxacin (91.43%) and amikacin (100%), respectively (Table 2).

E. coli isolates showed highest sensitivity against norfloxacin and ofloxacin (93.33% each) followed by amikacin and gentamicin (90% each), ciprofloxacin (83.33%), chloramphenicol (80%), tetracycline (70%), and enrofloxacin (66.66%). Higher sensitivity of E. coli isolates observed against these antibiotics, which might be attributed to their rare use in the treatment of gastrointestinal infections. However, highest resistance of E. coli isolates was observed against amoxycillin and erythromycin (100% each). Higher sensitivity of E. coli isolates against norfloxacin and ofloxacin (95.23% each) and highest resistance against amoxycillin and erythromycin (100.00% each) have been reported by Mishra et al. (2006), which are almost similar to our findings.

Hundred percent sensitivity of Salmonella spp. against each of amikacin, gentamicin and chloramphenicol and 100% resistance against each

<table>
<thead>
<tr>
<th>Bacterial isolates (number)</th>
<th>Amikacin</th>
<th>Amoxy-cillin</th>
<th>Norflo-xacin</th>
<th>Ciprofl-oxacin</th>
<th>Enroflo-xacin</th>
<th>Ofloxacin</th>
<th>Genta-micin</th>
<th>Erythro-myacin</th>
<th>Tetracycline</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (30)</td>
<td>27 (90)</td>
<td>0</td>
<td>28 (93.33)</td>
<td>25 (83.33)</td>
<td>20 (66.66)</td>
<td>28 (93.33)</td>
<td>27 (90)</td>
<td>0 (70)</td>
<td>21 (80)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Salmonella spp (2)</td>
<td>2 (100)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae (5)</td>
<td>4 (80)</td>
<td>0 (0)</td>
<td>3 (60)</td>
<td>4 (80)</td>
<td>4 (80)</td>
<td>5 (80)</td>
<td>4 (80)</td>
<td>2 (40)</td>
<td>2 (40)</td>
<td>3 (40)</td>
</tr>
<tr>
<td>Proteus ammoniae (12)</td>
<td>11 (91.66)</td>
<td>2 (16.66)</td>
<td>6 (50)</td>
<td>8 (66.66)</td>
<td>10 (83.33)</td>
<td>10 (83.33)</td>
<td>12 (100)</td>
<td>3 (25)</td>
<td>2 (16.66)</td>
<td>10 (83.33)</td>
</tr>
<tr>
<td>Campylobacter jejuni (4)</td>
<td>2 (50)</td>
<td>0 (0)</td>
<td>2 (50)</td>
<td>0 (0)</td>
<td>2 (50)</td>
<td>3 (75)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus (35)</td>
<td>30 (85.71)</td>
<td>20 (57.14)</td>
<td>20 (57.14)</td>
<td>33 (94.24)</td>
<td>32 (91.43)</td>
<td>30 (85.71)</td>
<td>30 (85.71)</td>
<td>15 (42.86)</td>
<td>15 (42.86)</td>
<td>30 (85.71)</td>
</tr>
<tr>
<td>Bacillus cereus (10)</td>
<td>10 (100)</td>
<td>0 (0)</td>
<td>7 (70)</td>
<td>8 (80)</td>
<td>5 (50)</td>
<td>8 (80)</td>
<td>8 (80)</td>
<td>4 (40)</td>
<td>2 (20)</td>
<td>5 (50)</td>
</tr>
</tbody>
</table>

Figures in brackets indicate percent.
of erythromycin and tetracycline were observed in our study. However, comparatively lower sensitivity of these isolates against gentamicin and chloramphenicol (75.00% each) has been reported by Chandra Shekhar and Sunil Kumar (2008).

*Klebsiella pneumoniae* isolates showed 100% sensitivity against ofloxacin. In contrast lower sensitivity (66.67%) against this antibiotic has been reported by Hussain et al. (2005). However, organism showed 60% resistance against amoxycillin, erythromycin and tetracycline.

Hundred percent sensitivity of *Proteus ammoniae* isolates were observed against gentamicin and lowest sensitivity of 16.66% against each of amoxycillin and tetracycline. However, comparatively lower sensitivity of this organism against gentamicin (82.40%) has been reported by Chandra Shekhar and Sunil Kumar (2008).

Highest sensitivity of *Bacillus cereus* isolates was recorded against amikacin (100%) with highest resistance against amoxycillin (100%). Hundred percent resistance of this organism against amoxycillin has also been reported by Hussain et al. (2005), which is similar to our finding.

*Campylobacter jejuni* isolates were found 75% sensitive against ofloxacin and 100% resistant against each of amoxycillin and ciprofloxacin.

*Staphylococcus aureus* showed highest sensitivity against ciprofloxacin (94.24%) and lowest with erythromycin and tetracycline (42.86% each). However, comparatively higher sensitivity of this organism against ciprofloxacin (100%) has been reported by Hussain et al. (2005).

**CONCLUSION**

The results of present study revealed that more than 50% of the milk samples exceeding the permissible limits of different microbial counts were found unfit for human consumption. Moreover, multiple drug resistance were also found with *E. coli*, *Salmonella* spp. and *Campylobacter jejuni* against some antibiotics, which is of public health concern and may pose serious problems in the treatment of those animals from which the milk samples were taken. The higher level of antibiotic resistance of some organisms might be due to the selection pressure exerted on the microorganisms. These resistant organisms may exist for longer periods in the population and cause public health hazards. To overcome these problems, there is need to maintain the good hygienic practices during milking, travel and distribution. Moreover, proper refrigeration of milk after its production, prevention of unhygienic water addition into the milk and adoption of high standards of personal hygiene may contribute a significant role in this regard.

**REFERENCES**


