ABSTRACT
A study was conducted to find out the physico chemical & bacteriological characteristics of Buffalo milk samples in Visakhapatnam district, Andhra Pradesh. The safety of milk is an important attribute for consumers of milk and dairy products. Milk and products derived from milk of dairy cows can harbor a variety of microorganisms and can be important sources of food borne pathogens.

The physicochemical assessment of milk is done by performing different tests like pH, colour, water test, clotting test, starch test, urea test, sugar test, salt test, formalin test, boric acid test, vanaspathi test, detergent test and ammonium sulphate test etc., the milk samples gave positive to the adulterants. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification. The final identification of resulted isolates was done by their biochemical testing mentioned in accordance to the Bergey’s Manual. The resulted bacterial isolates viz. *E.coli*, and *Staphylococcus*, are highly pathogenic. Poor quality of milk was recorded as the major risk factor for the alarming diseases.

KEYWORDS: Buffalo Milk, Adulterants, Quality assessment and Pathogenic bacteria.

INTRODUCTION
A highly nutritious liquid, milk is a very versatile kitchen ingredient. It is a white liquid produced by the mammary glands of mammals. Not only do animal milks vary significantly in terms of their nutritional content (although all are valuable sources of protein, calcium,
vitamins and minerals), but also milk from the same source can differ according to such factors as the animal’s diet, the time of milking, and the breed concerned (Ensminger, 1993). Milk contains many other nutrients and the carbohydrate lactose. As an agricultural product, milk is extracted from mammals during or soon after pregnancy and is used as food for humans. Fresh or raw milk as diet contributes to infants to adults in all over the world (Cousin 1982). Milk is enrichment medium to support growth of contaminating microbes. During transportation of milk at ambient temperature, the contaminated microbes may multiply and deteriorate the quality of loose milk (Schmidt and Van-Vleck, 1982). Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. These microorganisms are indicators of both the manner of handling milk from milking till consumption and the quality of milk. Milk produced under hygienic conditions from healthy animals should not contain >5X10^5 bacterial/ml (O’Connor 1994). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits et al; 2008). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley, 1990). The aim of the study is to evaluate the level of microbiological contamination of buffalo raw milk samples taken in Visakhapatnam area.

MATERIAL AND METHODS

In the present study, buffalo milk samples from five different places in Visakhapatnam district are collected in white plastic bottles, which were previously rinsed with distilled water and sterilized with 70% alcohol. At the collection point, the containers were rinsed thrice with the sample water before being used to collect the samples. The collected samples were placed in a thermocol box. Physic chemical analyses were done by using the standard procedures. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino, 2009). The final identification of resulted isolates was done by the biochemical tests in accordance to the Bergey’s Manual (Holt et al., 1984).

RESULT AND DISCUSSION

Physiochemical analysis of milk

Quality control tests for milk are very important to assure adulterant free milk for consumption. Adulteration of milk reduces the quality of milk and can even make it
hazardous. Adulterants like soap, acid, starch, table sugar and chemicals like formalin may be added to the milk. Most of the chemicals used as adulterants are poisonous and cause health hazards. Adulterants are mainly added to increase the shelf life of milk. Some of the preservatives like acid and formalin is added to the milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to the milk to increase the volume content of the milk. Some of the common adulterants found in milk and their detection are discussed.

pH
The pH value of milk was determined by using a digital pH meter. Prior to use, the pH meter was standardized with standard buffer solution of pH 4 and 7. pH of all the five samples are in the range of 6.5 – 6.8 (Table 1) which is slightly acidic in nature. Fresh cow milk has a pH of between 6.7 and 6.5. Values higher than 6.7 denote mastitis milk and values below pH 6.5 denote the presence of colostrum or bacterial deterioration. Because milk is a buffer solution, considerable acid development may occur before the pH changes. A pH lowers than 6.5 therefore indicate that considerable acid development has taken place. This is normally due to bacterial activity.

Clot-on-boiling test
Five ml of milk was placed in a test tube and it was placed in a boiling water bath for five minutes. Then, the test tube was carefully removed from the water bath and examined for the presence of floccules. All the four samples showed negative test but the sample B3 showed positive test (Table 1). The COB (clot on boiling test) positive in milk is due to high acidity (pH <5.8). High-acid milk should be rejected. The test allows you to identify colostral milk (which is produced in the first few days after parturition) or mastitic milk. Colostral milk should be rejected, because it has a very high percentage of whey proteins, which create problems when the milk is boiled or heated.

Water
The presence of water can be detected by putting a drop of milk on a polished slanting surface. The drop of pure milk flows slowly leaving a white trail behind it, whereas milk adulterated with water will flow immediately without leaving a mark. Percentage of water was absent in all the five samples (Table 1).
Starch
Add a few drops of tincture of iodine or iodine solution. Formation of blue colour indicates the presence of starch. (Iodine solution is easily available in the medical stores. All the five samples showed negative to the starch test (Table 1). Usually flour from wheat, corn, rice, tapioca is the general starch adulterants to increase fat content and mask adulteration. They reduce nutritive value.

Urea
Take a teaspoon of milk in a test tube. Add ½ teaspoon of soybean or arhar powder. Mix up the contents thoroughly by shaking the test tube. After 5 minutes, dip a red litmus paper in it. Remove the paper after ½ a minute. A change in colour from red to blue indicates the presence of urea in the milk. All the five samples showed negative to the urea test (Table 1). Urea is added in synthetic milk to raise the fat value. It damages the intestinal tract and digestive system.

Detergent
Shake 5-10 ml of sample with an equal amount of water. Lather indicates the presence of detergent. All the five samples showed negative to the detergent test (Table 1). Detergent increases the fat value and mask adulteration with water with ditto health hazards. Soap is added to milk to increase the foaming of milk and thus to have thick milk. Addition of such chemicals will cause health problem especially related to stomach and kidneys.

Synthetic Milk test for protein
The milk can easily be tested by urease strips (available in the medical store. Colour chart of the urease strip test given below will show the quantity of urea present in milk. All the five samples showed positive to the synthetic milk test for protein (Table 1). The positive test indicates the presence of milk powder in the test sample. This indicates the mask adulteration which reduces nutritive value.

Test for glucose / Invert Sugar
Take a strip of diacetic strip and dip it in the milk for 30 sec – 1min if the strip changes colour, than it shows that the sample of milk contains glucose. If there is no change in colour of the strip, than glucose is absence. All the five samples showed positive to the invert sugar test (Table 1). If it is made synthetically by adding colour, water, paint, oils, alkali, detergent etc. Glucose/ inverted sugar syrup is added to milk to increase consistency and test.
Sugar
Take 3 ml of milk in a test tube. Add 2 ml of the hydrochloric acid. Heat the test tube after adding 50 mg of resorcinol. The red colouration indicates use of sugar in the milk. All the samples showed positive to sugar test (Table 1). Sugar is mixed to increase the lactometer reading to mask dilution with water. Chances of getting epidemic diseases are high if the water is bad.

Boric Acid
Take 3 ml of milk in a test tube. Add 20 drop of hydrochloric acid and shake the test tube or mix up the content thoroughly. Dip a yellow paper strip, and remove the same after 1 min. A change in colour yellow to red, followed by the change from red to green, by addition of 1 drop of ammonia solution, indicates that the boric present in milk. All the samples showed negative to boric acid test (Table 1). Boric acid was believed to "purify" milk, removing the sour taste and smell from milk. Small amounts of boric acid can cause nausea, vomiting, abdominal pain and diarrhoea.

Vanaspathi
Take 3 ml of milk in a test tube. Add 10 drops of hydrochloric acid. Mix 1 teaspoon full of sugar. After 5 min, examine the mixture. The red coloration indicates the presence of vanaspati in the milk. All the five samples didn’t show vanaspathi in the milk. Vanaspathi is mixed to increase the fat content in the milk thus milk appear rich in fat content. The vanaspati accumulates the trans fat which in turn increase the bad cholesterol level which results in awful diseases like heart attack.

Formalin
Take 10ml of milk in a test tube and add 5 ml of concentrated sulphuric acid from the side of the wall without shaking if a violet or blue ring appears at the intersection of two layers than it shows presence of formalin. All the five samples showed negative to the formalin test (Table 1) because it usually added to pasteurised milk to retain its freshness and to increase its shelf life. Formalin is a human carcinogen listed by the International Agency for Research on Cancer. It is a preservative and can preserve milk for long period of time. Due to its high toxicity, it is considered to cause liver and kidney damage. It reacts with Sulphuric acid and ferric chloride to give a purple colour ring at the junction of the milk layers, thereby indicating the presence of formalin adulterated in milk.
Ammonia Sulphate
Take 5 ml of hot milk in a test tube. Add a suitable acid, Ex: Citric Acid. The Whey obtained is separated & filtered. Take the Whey in another test tube & add 0.5ml of 5% barium chloride. Appearance of participate indicates the presence of ammonium sulphate. Take 5 ml of milk in a test tube. Add 2.5ml of 2% sodium hydroxide, 2.5 ml of 2% sodium hypo chloride And 2.5 ml of 5% phenol solution. Heat the solution for 20 sec in boiling water bath. If bluish colour turns to deep blue, it indicates the presence of ammonium sulphate. However in case it turns pink, it shows that the sample is free from ammonium sulphate. All the five samples showed negative to the ammonium sulphate test (Table 1). Ammonium Sulphate is added to the milk as it increases the lactometer reading by maintaining the density of milk. Ammonium sulphate adulterated milk can be detected by adding sodium hydroxide, sodium hypochlorite and phenol, the reaction of the three reagents with ammonium sulphate results in formation of deep blue colour. The deep blue color is generated when the amine reacts with phenol in the presence of hypochlorite in an alkaline environment, results in the formation of a complex which is blue in colour.

Salt
Take 5 ml of silver nitrate reagent in a test tube. Add 2-3 drop of potassium dichromate reagent. Add 1 ml of milk in the above test tube and mix thoroughly. If the contents of the test tube turn yellow, then milk contains salt. If it turns to chocolate the pH meter was standardized with standard buffer solution of pH 4 and 7. Colour or reddish brown, the milk sample is free from salt. B₁ and B₃ samples showed positive to the salt test (Table 1). Salt is added to tweak lactometer reading when milk is adulterated with water. This reduces the nutritive value.

Colour
The colour will be observed by appearance of the milk. All the four samples appear in white colour B₃ in yellowish white in colour which may be due to high addition of whey protein to increase the lactometer reading (Table 1).

Microbiological Analysis
Milk is a good medium for the growth of microorganism. A variety of microorganism can be found in raw milk. It may contain some harmful microorganisms like bacteria along with some potentially beneficial microbes. Microbiological analysis of milk is carried out to determine the degree of bacterial contamination in milk and to understand the chemical
changes brought in milk as a result of microbial action. Contaminated milk is one of the important sources for transmission of diseases from animals to humans. The main reason for this contamination is the un-proper handling of milk. Normally milk is contaminated during the milking process by the microorganisms present in the exterior surface of the animals, pipelines such as udder and adjacent areas. Unsterilized dairy utensils such as milking machines, milk cans are also a good source of contamination by the microorganism.

MILK PHOSPHATE TEST
This is a statutory test. The test can be used to assess the quality of milk i.e., the extent of microbial contamination and as a measure for the total inactivation and of the enzyme along with the pathogenic microorganism at 145°F for 30 minutes or 160°F for 15 seconds as in the recognized methods of pasteurization. The presence of the enzyme is detected based on its ability to catalase the liberation of phenol from disodium phenol phosphate. The phenol is estimated calorimetrically with FC reagent, which yields a blue coloured complex. The amount of phenol liberated is proportional to enzyme present in it which in turn indicates the extent of microbial population which in turn indicates the extent of microbial population. All the milk samples showed positive towards the milk phosphate test.

Methylene Blue Test (MBRT)
Methylene blue test for the assessment of mastitis was performed according to procedure described by method followed by Awan, J.A and S.U.Rehman. The test is used to diagnose mastitis, the ability of bacteria to reduce the colour of methylene blue dye from the milk sample. Dye reduction time is inversely proportion to the presence of total number of bacteria in sample, hence greater the bacterial population shorter the dye reduction time. Except the milk sample from Sabbavaram all the milk gave poor results in MBRT test which mean it contains l more microbial load (Table 2).

Determination of the most probable number of bacteria coliform
This is done to Milk confirm whether it contains lactose-fermenting gas producing bacteria. It is used to determine the most probable number (MPN) of coliforms in a sample of milk besides their properties of fermenting lactose and producing gas. If after inoculation and incubation of lactose-broth, gas is produced, it is presumed that coliforms are present in the sample.
The data regarding the total Most Probable Number (MPN) has been present in Table 4. The data shows highly significant results. The higher value (75) of MPN were observed in B1, B2, B3 followed by B4 (21) and the lowest value (0) of MPN were observed in and B5. The MPN positive indicates the presence of faecal coliform *E. coli* and *Staphylococci* in the milk sample. The correlation of the number of coliforms with total bacteria was understandable, because the coliforms represented a part of the total bacterial count. From the same reason there were also correlations between the number of coliform and psychrotropic microorganism because a lot of coliform bacteria are capable to growth at low temperatures.

**Determination of Coliforms**

Coliform counts were determined by pour plate method on Eosin Methylene Blue Agar, prepared according to the manufacturer’s instructions. All plates were incubated at 37°C for 24 hours.

Coliform bacteria have minimum generation time and multiply at rapid rate to reach its number up to Unhygienic level. Coliforms such as *E. coli* and other Gram negative bacteria (pseudomonas spp.) are also common on the dairy sheds. Increased number coliform count in milk could be due to contamination with faecal and bedding material. Pasteurized milk shouldn’t contain any coliform bacteria as though coliform bacteria can’t survive the pasteurization temperature but the presence of TCC (Total coliform count) of the pasteurized milk samples indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials (Srairi et al, 2006), defects in pipe lines. A coliform count more than 100 cells/ml suggests poor hygienic practices (Jayarao and Wolfgang, 2003). Higher Coliform counts were reported in many countries; Khan et al. (2008) reported a count between 300- 400 cell/ml, lower than counts of more than 600 cell/ml reported in the summer market milk. Mutukumiram (1996) calculated a higher rate ranging between 3200 to 23000 cell/ml. Count of 1000 cell/ml was reported by Saitanu et al. (1996); and Shojaei and Yadollahi (2008) estimated a range between 1000 to 1300 cell/ml. During this study the percentage of the highest count of more than 1100 cell/ml was higher in summer (17.1%) compared to 8.4% during winter. while this count was higher in Omdurman (19.0%), followed by Khartoum (18.4%) then Khartoum North which was 17.1% but the differences between the Coliform counts in the three regions were statistically insignificant.
**Total Bacterial Count (TBC)**

Total bacterial count in different milk samples was determined by method followed by Esron et al. Count the microbial population in sample as number of microscopic fields present in 1 cm square prescribed areas of microscope glass slides. The data regarding the total bacterial count (TBC) has been present in Table 3. The data shows highly significant results. At $10^{-3}$ dilutions the higher value of TBC were observed in B$_2$ (512 cfu’s) followed by B$_3$ (463 cfu’s), B$_4$ (425 cfu’s), B$_1$ (62 cfu’s) and lowest load was observed in B$_5$ (24 cfu’s). At $10^{-4}$ dilutions the higher value of TBC were observed in B$_4$ (236 cfu’s) followed by B$_3$ (176 cfu’s), B$_2$ (133 cfu’s), B$_1$ (11 cfu’s) and lowest load was observed in B$_5$ (7 cfu’s).

The high counts may also due to miss handling during milking, animal bedding and by mixing of abnormal milk in good quality milk. The reason for high bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers (Monika Saxena & Poonam Rai 2013). The presence of bacteria in milk might be due to many factors including the milk quality, sanitation of process plant, status of packaging material and also the handling process (Tekinsen et al., 2007).

The microbial count in the milk sample is may be due to the abscess in the udder tissue. An abscess is an abnormal cavity containing pus. Formation of abscesses in animals is very common. All the species of animals were affected with abscess in different areas of body like yoke region, liver, umbilicus, conjunctiva etc. but abscess forming in and around udder tissue are rare. Abscesses commonly develop after bite wounds, scratches, or when objects penetrate the skin and then the skin heals over. Usually cattle bit the infected area this may increase the severity and the other reason is the water borne pathogens may affect the wound area thus responsible for abscess formation. Different bacterial species like *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* were isolated as causative organism of udder abscess (G Kamalakar et al., 2014).

**Yeast and Mould Count**

In three milk samples i.e., B$_1$, B$_2$ & B$_3$ mould species were found, those are Mucor, Fusarium and Pencillium. A few genera of moulds were usually found in raw milk samples, so it could be expected that the feed was one of the possible sources of contamination of raw milk in spite of Finne Kure et al., (2004) adduced proofs that there are many possible sources of contamination of raw milk, beside the feed also the air and the environment.
Table 1: Physico-chemical analysis of the milk sample

<table>
<thead>
<tr>
<th>S.no</th>
<th>Name of the sample</th>
<th>Water</th>
<th>Starch</th>
<th>Urea</th>
<th>Detergent</th>
<th>Synthetic milk test for protein</th>
<th>Test for glucose/invert sugar</th>
<th>Vanaspathi</th>
<th>Formalin</th>
<th>Ammonium sulphate</th>
<th>Salt</th>
<th>Sugar</th>
<th>Boric acid</th>
<th>Colour</th>
<th>pH</th>
<th>Clot on boiling test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffalo milk from Allipuram</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>White</td>
<td>6.5</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo milk from Kommadi</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>White</td>
<td>6.8</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>Buffalo milk from Rambilli</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>Yellowish White</td>
<td>6.8</td>
<td>p</td>
</tr>
<tr>
<td>4</td>
<td>Buffalo milk from Gottiwada</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>White</td>
<td>6.8</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>Buffalo milk from Buffalo milk from Sabbavaram</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>White</td>
<td>6.5</td>
<td>A</td>
</tr>
</tbody>
</table>
Table 2: Methylene blue test (MBRT)

<table>
<thead>
<tr>
<th>S.no</th>
<th>Name of the Samples</th>
<th>Decolourisation Time</th>
<th>Quality of milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>Buffalo milk from Allipuram</td>
<td>30 min</td>
<td>Very poor</td>
</tr>
<tr>
<td>B₂</td>
<td>Buffalo milk from Kommadi</td>
<td>30 min</td>
<td>Very poor</td>
</tr>
<tr>
<td>B₃</td>
<td>Buffalo milk from Rambilli</td>
<td>2 hour</td>
<td>Poor</td>
</tr>
<tr>
<td>B₄</td>
<td>Buffalo milk from Gottiwada</td>
<td>6 hour</td>
<td>Fair</td>
</tr>
<tr>
<td>B₅</td>
<td>Buffalo milk from Sabbavaram</td>
<td>8 hour</td>
<td>Good</td>
</tr>
</tbody>
</table>

Table 3: Enumeration of microorganisms in different milk samples by standard plate count methods

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Total plate count 1/1000</th>
<th>Total plate count 1/10000</th>
<th>MPN/100ml Total coliform</th>
<th>E.coli count</th>
<th>Staphylococcal count</th>
<th>Yeast and Moulds count</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>62</td>
<td>11</td>
<td>75</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>B₂</td>
<td>512</td>
<td>133</td>
<td>75</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>B₃</td>
<td>463</td>
<td>176</td>
<td>75</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>B₄</td>
<td>425</td>
<td>236</td>
<td>21</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>B₅</td>
<td>24</td>
<td>7</td>
<td>-</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

CONCLUSION

The quality of milk produced in the study area was poor. This was evident from the higher values of total bacterial count (TBC), coliform count (CC) present in the milk samples. Handling and transportation also involved in microbial growth. The addition of impure water in milk may play vital role to enhance the count population. Bacterial growth increased rapidly and finally quality of milk reached at un-acceptable level. Consumption of lower quality milk may lead to serious human health problems. The consumers must be more active against milk adulteration going on in the city. It is important to have a quality control system that regularly check and ensure that only good quality milk is sold.

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