RESEARCH ARTICLE

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# Bacterial load of water used for livestock's operations in Al Obied city of North Kordofan State, Sudan

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***KEYWORDS***

Bacterial load
Coliform
Dairy farm
Water

***ABSTRACT***

This study was conducted in Al Obied city in North Kordofan State in order to evaluate the bacterial load in water for livestock operations, based on bacteriological examinations and viable counts. A total of 40 water samples were obtained (10 from dairy farms, 14 from animal's markets and 16 from slaughterhouses). All samples were cultured on Blood Agar and MacConkey for bacterial isolation and on nutrient agar for viable counts. The result of bacterial viable count of water samples collected from different sources was high and ranging between $7X10^8$ and $36X10^8$ CFU/ml. Application of analysis statistic using one way ANOVA for bacterial count in water collected from different sources, revealed that there was statistical significance (p. value= 0.000, p-value< 0.05) for water samples collected from different sites. Bacterial isolates from dairy farms comprised 10 isolates including 3 *Escherichia coli* (25.1%), 2 *Klebsiella pneumoniae* (16.7%), 2 *Pseudomonas aerogenosa* (16.7%), 1 *Staphylococcus aureus* (8.3%), 1 *Streptococcus uberis* (8.3%), 1 *Bacillus subtilis* (8.3%), 1 *Micrococcus variens* (8.3%) and 1 *Micrococcus luteus* (8.3%). Gram negative Bacterial isolates represented the higher percentage (58.5%) of the total bacterial isolates from dairy farms. Bacteria isolated from animals' markets were 5 *E. coli* (31.3%), 4 *Ps. aerogenosa* (25.0%), 4 *S. aureus* (25.0%), 2 *Str.uberis* (12.5%), 1 *B. subtilis* (6.2%). Gram negative Bacteria represented the higher percentage (56.3%) of the total bacteria isolated from animals' markets. Bacteria isolated from slaughterhouses were 5 *S. aureus* (31.3%), 4 *Ps. aerogenosa* (25.0%), 3 *Str. uberis* (18.7%), 2 *M. variens* (12.5%),2 *M. luteus* (12.5%). Gram positive Bacteria represented the higher percentage (75.0%) of the total bacteria isolated from slaughterhouses.

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## INTRODUCTION

Animals and humans can survive days, weeks or months without food, but only about four days without water (WHO, 2008). Water, although an absolute necessity for life can be a carrier of many diseases. Water can be hard or soft, natural or modified, bottled or tap, carbonated or still (Kendall, 1992). Good quality water is odorless, colorless, tasteless and free from fecal pollution and chemicals in harmful amounts. The World Health Organization (WHO) has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water, or unavailability of water (WHO, 2008). Microorganisms enter into drinking water via humans and animals' intestinal secretions in areas where sanitation conditions are poor or absent. When found in drinking water, microorganisms constitute a real indication that it should not be used for human consumption if these contaminants are found in excess of the maximum permissible level ($1\times10^2$ CFU ml-1) (WHO, 2008). Bacterial contamination can get into groundwater by many ways; wild and domestic animals, birds and dairy farms wastes situated in a watershed area or within the hydrological catchments of groundwater. However, these have been found to be a pathogenic contamination source of drinking water (Obiri and Jones, 2001). Also, the presence of campylobacter in waters within agricultural areas is a real evidence of environmental contamination by sewage effluent coming from agricultural areas (Obiri and Jones, 2001). Biomass that resulted from degradable materials are deposited into drinking water distribution pipes and accumulates biofilms which accelerate the growth of microorganisms and protect them against disinfection agents (Lewis, 2001). A major type of bacteria in polluted water is coliform bacteria. The most important species of the group includes *E. coli, klebsiella spp. and Enterobacter spp.* (Lewis, 2001). Non-coliform bacteria are also common in polluted water and include *Streptococcus, Proteus and Pseudomonas spp. Bifidobacteria* are one of the most common bacterial types found in the intestines of humans and other animals and may be used as indicators for human fecal pollution (Bonjoch *et al.*, 2004). In Sudan Aliaa (2005) isolated coliforms from drinking water samples collected from Khartoum State. Amna and Atif (2014) isolated *Micrococcus spp., Pseudomonas spp., Bacillus spp., Staphylococcus spp., Corynobacterium spp., Aeromonas spp., Actenobacillus spp., Moraxella spp. and Flavibacterium spp*. Regarding bacterial counts, application of analytical statistic using one-way analysis of variance (ANOVA) revealed that there was no statistical significance (F-value = 0.198, p-value > 0.05) for bacterial counts of the drinking water in Khartoum State (Amna and Atif, 2014).

This study was aiming at evaluating the bacterial load of water in livestock operations in Al Obied city in North Kordofan State.

## MATERIALS AND METHODS

### *Source of samples*

A total of 40 water samples were collected from different sources (tap and tank water) in Al Obied city, North Kordofan state during the last 6 months of the year 2019.

### *Sampling procedure*

Fifty milliliters of tap water were collected in glass bottles. Collection was done as follows:

(1) The outside nozzle of the tap was cleaned carefully.

(2) The tap was turned on full, and the water was allowed to run to waste for 1 minute.

(3) The sample bottle was then filled from the gentle flow of water.

(4) Contamination was avoided by not allowing any surface to touch the screw thread of the bottle neck or the inside of the cap.

(5) The cap of the bottle was then replaced.

Fifty milliliters of tank water were collected in glass bottles. Collection was done as follows:

(1) The cap was removed and the mouth of the bottle was faced up.

(2) The bottle was pushed forward horizontally until it was filled.

The samples from taps and tanks were labeled with a sample code number, and transported at 4°C to the laboratory of college of Veterinary Medicine, University of Bahri.

### *Bacterial viable count*

According to Quinn et al. (2011), tenfold dilutions of a bacterial suspension was made before conducting a viable count to find the number of bacteria/ml in the original sample. Sample was thoroughly mixed before sampling and a separate pipette was used for each transfer step. Serial dilutions of water samples were made. Spread plate method was followed and an inoculum of 0.1 ml of each dilution was placed on the surface of an agar plate. The inoculum was spread rapidly over the entire agar surface. Inoculated plates were left to dry and then incubated for 24–48 hours at 37C. The total colony count per milliliter of water was calculated by multiplication of the number of colonies counted by dilution marked. After incubation, plates inoculated with a sample dilution yielding between 30 at 300 colonies are read; the colony count should be an average of the two or four plates inoculated with the selected dilution.

### *Isolation, identification and characterization of bacterial isolates*

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth streaked onto blood agar, MacConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37$^{o}$C for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in carbohydrates media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4$^{o}$C.

### *Biological and biochemical identification of the bacteria*

The purified isolates were identified as previously described by Smith et al. (1986) and Barrow and Feltham (2004). The identification includes: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test and fermentation of carbohydrates).

## RESULTS

### *Bacterial viable count of water samples collected from dairy farms in Al Obied city*

All of the 10 water samples collected from dairy farms had a bacterial viable count ranging between 20X10$^{8}$ and 36X10$^{8}$ CFU/ml (Table 1).

**Table 1:** Bacterial viable counts of water samples collected from dairy farms in Al Obied city.

| Sample number | CFU/ml | Sample number | CFU/ ml |
|---|---|---|---|
| 1 | 35X10$^{8}$ | 6 | 35X108 |
| 2 | 34X10$^{8}$ | 7 | 35X10$^{8}$ |
| 3 | 35X10$^{8}$ | 8 | 35X10$^{8}$ |
| 4 | 28X10$^{8}$ | 9 | 28X10$^{8}$ |
| 5 | 36X10$^{8}$ | 10 | 20X10$^{8}$ |

### *Bacterial viable count of water samples collected from animals' markets in Al Obied city*

All of the 14 water samples collected from animals' markets had a bacterial viable count ranging between 7X10$^{8}$ and 35X10$^{8}$ CFU/ml (Table 2).

### *Bacterial viable count of water samples collected from slaughterhouses in Al Obied city*

All of the 16 water samples collected from slaughterhouses had a bacterial viable count ranging between 8X10$^{8}$ and 35X10$^{8}$ CFU/ml (Table 3).

### *Comparison between bacterial viable counts of water from different sources*

Application of analytical statistic using one-way ANOVA revealed that there was statistical significant (p-value= 0.000, p-value< 0.05) for bacterial viable counts of water collected from different sites.

**Table 2:** Bacterial viable counts of water samples collected from animals' markets in Al Obied city.

| Sample number | CFU/ml | Sample number | CFU/ml |
|---|---|---|---|
| 1 | 18X108 | 8 | 13X108 |
| 2 | 19X108 | 9 | 20X108 |
| 3 | 23X108 | 10 | 11X108 |
| 4 | 20X108 | 11 | 7X108 |
| 5 | 23X108 | 12 | 10X108 |
| 6 | 13X108 | 13 | 9X108 |
| 7 | 35X108 | 14 | 8X108 |

**Table 3:** Bacterial viable counts of water samples collected from slaughterhouses in Al Obied city.

| Sample number | CFU/ml | Sample number | CFU/ml |
|---|---|---|---|
| 1 | $28X10^8$ | 9 | $34X10^8$ |
| 2 | $25X10^8$ | 10 | $35X10^8$ |
| 3 | $29X10^8$ | 11 | $20X10^8$ |
| 4 | $28X10^8$ | 12 | $16X10^8$ |
| 5 | $33X10^8$ | 13 | $24X10^8$ |
| 6 | $19X10^8$ | 14 | $9X10^8$ |
| 7 | $18X10^8$ | 15 | $8X10^8$ |
| 8 | $20X10^8$ | 16 | $10X10^8$ |

### *Bacteria isolated from water samples collected from dairy farms in Al Obied city*

In this investigation a total of 12 bacterial isolates were obtained from 10 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 3 *E. coli* (25.1%), 2 *Klebsiella pneumoniae* (16.7%), 2 *Pseudomonas aerogenosa* (16.7%), 1 *Staphylococcus aureus* (8.3%), 1 *Streptococcus uberis* (8.3%), 1 *Bacillus subtilis* (8.3%), 1 *Micrococcus variens* (8.3%),1 *Micrococcus luteus* (8.3%) (Table 5).

### *Bacteria isolated from water samples collected from animals' markets in Al Obied city*

In this investigation a total of 16 bacterial isolates were obtained from 14 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 5 *E. coli* (31.3%), 4*Ps. aerogenosa* (25.0%), 4*S. aureus* (25.0%), 2*Str.uberis* (12.5%), 1 *B. subtilis* (6.2%) (Table 6).

### *Bacteria isolated from water samples collected from slaughterhouses in Al Obied city*

In this investigation a total of 16 bacterial isolates were obtained from 16 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 5*S. aureus* (31.3%), 4*Ps. aerogenosa* (25.0%), 3*Str.uberis* (18.7%), 2*M. variens* (12.5%),2*M. luteus* (12.5%) (Table 7).

## DISCUSSION

Microbiological quality analysis of drinking water in dairy farms, animal markets and slaughter houses is of paramount concern because of the possible risk to health caused by bacteria in drinking water. Monitoring and assessment of quality of drinking water is primarily a health-based activity which helps to protect public health Lack of basic knowledge affects clearly the quality of water and this could strongly result in water borne disease (Al Beeli, 2006).

In this study the bacterial viable count of water samples collected from dairy farms, animal markets and slaughterhouses in Al Obied city, was high and ranging between $7X10^8$ and $36X10^8$ CFU/ml. According to WHO (2008) microorganisms should not exceed the maximum permissible level ($1 \times 10^2$ CFU $ml^{-1}$) in water used for consumption. Also, clean water is important for animals' health and many pathogenic bacteria can be transmitted through water (peter *et al.*, 2017). Amna and Atif (2014) mentioned that the total viable count for bacteria showed that water samples collected from Khartoum State were found most loaded; this may be logical because troughs are exposed to contamination

**Table 4**: Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

| **Test** | ***E. coli*** | ***S. aureus*** | ***Ps. aerogenosa*** | ***K. pneumoniae*** |
|---|---|---|---|---|
| Aerobic growth | + | + | + | + |
| Colonies on MacConkey | Bright Pink | Pink | Bright Pink | Pink |
| Haemolysis on blood agar | + | + | + | - |
| Gram reaction | - | + | - | - |
| Shape | Rods | Cocci | Rods | Rods |
| Spore | - | - | - | - |
| Motility | + | - | + | - |
| Catalase | + | + | + | + |
| Oxidase | - | - | + | - |
| Indole | + | - | - | + |
| Methyl red | + | + | - | - |
| VP | - | - | - | - |
| Citrate | - | - | + | + |
| $H_2S$ | - | - | - | - |
| O/F | + | + | + | + |
| Glucose | + | + | - | + |
| Lactose | + | + | - | + |
| Coaggulase | - | + | - | + |

**Table 4 (continued):** Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

| **Test** | ***Str. uberis*** | ***M. luteus*** | ***M. variens*** | ***B. subtilis*** |
|---|---|---|---|---|
| Aerobic growth | + | + | + | + |
| Colonies on MacConkey | Pink | Pink | Pink | Pink |
| Haemolysis on blood agar | - | + | - | + |
| Gram reaction | + | + | + | + |
| Shape | Cocci | Cocci | Cocci | Rods |
| Spore | - | - | - | + |
| Motility | - | - | - | + |
| Catalase | - | + | + | + |
| Oxidase | - | + | + | - |
| Indole | - | - | - | - |
| Methyl red | - | - | - | - |
| VP | + | - | - | - |
| Citrate | - | - | - | - |
| $H_2S$ | - | - | - | - |
| O/F | + | + | + | + |
| Glucose | + | + | + | + |
| Lactose | + | + | + | + |
| Coaggulase | - | - | - | - |

from many sources like cattle while drinking, animal faeces, air, dust and feed stuffs, similarly from bacterial contamination and bad storage of water.

In this study different bacterial species (*E. coli*, *K. pneumoniae*, *Ps. Aerogenosa*, *S. aureus*, *M. variens*, *M. luteus*, *Str. uberis* and *B. subtilis*), were isolated from water samples collected from different sources in Al Obied city. Amna and Atif (2014) reported that *Micrococcus spp*., *Pseudomonas spp.*, and Bacillus spp. were dominant in water samples collected from dairy farms in Khartoum State. Sanaa and Rawda (2009) isolated faecal coliform (*E. coli*), coliform group (*Klebsiella spp., Citrobacter spp., Enterobacter spp.*), some pathogenic and potential pathogenic bacteria (*S. aureus*, Salmonella spp., *Yersienia enteriocolitica*, *Proteus spp*., *Bacillus spp.* and *Pseudomonas aeruginosa*). Isolation of pathogenic bacteria is of highly importance and indicated that the water is unsafe.

In this study coliforms were predominant (51.5% and 56.3% in dairy farms and animal markets respectively) bacteria compared to gram positive bacteria. Also, in this study faecal coliorm (*E. coli*) represented the predominant bacteria (25.1% and 31.3% in dairy farms and animal markets respectively). Sara *et al*. (2016) reported that most water samples obtained from Khartoum state showed the presence of total coliform, and fecal coliform. In addition, Amira (2014) reported that water samples collected from Wadmedani were highly contaminated with total coliform and fecal coliform compared to Khartoum drinking water samples. In relevance to our findings, Al-Beeli (2006) reported that *E. coli* is the predominant bacteria (18%) isolated from water samples collected from dairy farms in Eastern and Southern Sudan. Also Mohamed *et al*. (2013) reported high levels of total coliform in water samples collected from dairy farms in West Kordofan. Workers in dairy farms, animal markets and slaughterhouses in Al Obied city are at risk of infection with bacterial diseases transmitted by water because of their bad practices as washing their hands with animals' water. Also, meat consumers will be at risk after washing carcasses with unsafe water.

**Table 5:** Total number and percentage of bacteria isolated from water samples collected from dairy farms in Al Obied city.

| Isolated bacteria | No. / % |
|---|---|
| *E. coli* | 3 (25.1%) |
| *K. pneumoniae* | 2 (16.7%) |
| *Ps. aerogenosa* | 1 (5.6%) |
| *Str. uberis* | 2 (16.7%) |
| *S. aureus* | 1 (8.3%) |
| *M. luteus* | 1 (8.3%) |
| *B. subtilis* | 1 (8.3%) |
| **Total** | **12 (100%)** |

**Table 6:** Total number and percentage of bacteria isolated from water samples collected from animals' markets in Al Obied city.

| Isolated bacteria | No. / % |
|---|---|
| *E. coli* | 5 (31.3.1%) |
| *Ps. aerogenosa* | 4 (25.0%) |
| *Str. uberis* | 2 (12.5%) |
| *S. aureus* | 1 (6.2%) |
| *B. subtilis* | 1 (6.2%) |
| **Total** | **16 (100%)** |

**Table 7:** Total number and percentage of bacteria isolated from water samples collected from slaughterhouses in Al Obied city.

| Isolated bacteria | No. / % |
|---|---|
| *S. aureus* | 5 (31.3.0%) |
| *Ps. aerogenosa* | 4 (25.0%) |
| *Str. uberis* | 3 (18.7%) |
| *M. variens* | 2 (12.5%) |
| *M*. luteus | 2 (12.5%) |
| **Total** | **16 (100%)** |

## CONCLUSION AND RECOMMENDATIONS

Regarding bacterial count, the results showed high level of contamination of all water samples collected from dairy farms, animals' markets and slaughterhouses in Al Obied City.

Application of analysis statistic using one-way ANOVA revealed that there was statistical significant (p.value = 0.000, p-value< 0.05) for water sample collected from different sites. Coliforms (*K. pneumoniae*, *Ps. Aerogenosa*) and faecal coliorm (*E. coli*) were predominant bacterial isolates.

Microbiological analysis of water for total bacteria and coliform is necessary to determine sanitary quality. The possible consequence is of such severity than its control which is always very important and should never be compromised.

Water analysis for the detection of faecal pollution should be prompted to determine the level of faecal pollution in ground water resources whenever water is intended for animal and human use.

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