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MICROBIAL CONTAMINATION OF WATER STORED IN EARTHEN POTS WITHIN UNIVERSITY OF AGRICULTURE ENVIRONS

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ABSTRACT

Microbial analysis of water stored in different earthen pots was carried out in Iorkume, Shamija and Leva-Amor villages which are amongst the villages that make up the host communities of University of Agriculture Makurdi. Sixty (60) samples of water obtained from various sources such as University of Agriculture Water Works, streams and wells were collected randomly from the three villages studied for identification and characterization of possible microbial contaminants and to determine its suitability for domestic uses. The viable counts of the samples analyzed ranged between $1x10^{-6}$ and $18x10^{-6}$ cfu/ml. Nine (9) isolates were identified from the samples which include: *Staphylococcus aureus* 33 (55%), *Escherichia coli* 65 (108%), *Bacillus spp.* 20 (33%), *Klebsiella spp.* 12 (20%) *Mucor spp.* 36 (60%) and *Penicilliun spp.* 19 (32%), *Pseudomonas* spp 23(38%), *Rhizopus spp.* 54(90%), *Candida spp.* 23(38%) on average. The presence of these organisms indicates a considerable level of microbial contamination of water stored in earthen pots in the three villages studied. Improvement in the basic hygienic practices in acquisition and storage of water in these pots for domestic use and the medical implications of the organisms identified is hereby suggested.

Keywords: Microbial contamination, Micro-organisms, Isolation, Disease

INTRODUCTION

One of the main components necessary for providing safe drinking water is the ability to safely store it in homes of users that do not have pipe borne water supply at all times. Monitoring and detection of indicator and disease-causing microorganisms in water are major role of every consumer of water. (Prescott *et al.,* 2008).Water is an important constituent of all forms of life and clean, uncontaminated water is particularly required to maintain health. The drinking

water of most communities and municipalities is obtained from surface water such as rivers, streams and lakes, such natural water supplies particularly stream and rivers are likely to be polluted with domestic and industrial waste (Pelczar *et al.,* 1986).The world health organization (WHO, 1985), stated that the contamination of drinking water by human and animal waste constitutes the most common mechanism for transmission of enteric pathogens to human directly through food preparation. The pathogens must prominently transmitted through water are those which cause infectious of the intestinal tract, namely typhoid and paratyphoid fevers, dysentery and cholera. The causative organisms of these diseases are present in feaces or urine and infected persons when discharged gain entrance into the body of waters that ultimately serves as a source of drinking water. (Bitton and Gerba, 1984).Maintaining a safe drinking water remains essential to human health a living organisms required a wide variety of organic compounds for growth, repair, maintenances and reproduction, water is the most important as well as one of the most abundant of those organic compounds and it is particularly vital to living organisms (Tortora *et al*., 2002).Earthen pots like other tanks are storage containers which are usually used for storing water for human consumption, irrigation in Agriculture, fire suppression, livestock use and domestic uses. The use of earthen pots for water storage is as old as civilization (Hill, 1983).The people of Nyanza province of Kenya, located along the shores of lake Victoria, have a tradition of storing their water in wide-mouth earthen pots preferable because of the evaporative cooling effect and the clay makes the water palatable for drinking (Bovin and Morohashi; 2004). The pots has a wide mouth which encourages drawing of water with cups and often, the hands holding the cups are contaminated and also according to Ahmed et al (1998), in Bangladesh, did find evidence of bacterial growth on the internal surface of the base of the storage container (earthen pots). So, even distilled water if stored improperly can easily be contaminated, leading to incidence of diarrhea diseases and other water borne diseases. Because it has been reported that over 50% of the outbreak of water borne diseases results from consumption of contaminated storage water hence it has become worthwhile venture to investigate into monitoring the microbial quality of storage water (Bittton and Gerba, 1984).

Objective of the study are:

- To identify the microorganisms associated with water stored in earthen pot and to characterize them.
- To determine if the quality of this stored water in earthen pots attains the maximum hygienic requirement for domestic uses.

- To proffer useful suggestion if any for further investigation into the shape, size and fetching mechanism of this storage container (earthen pot).

SAMPLE COLLECTION

A total of 60 samples, 20 each in the three villages Shamija, Iorkume and Leva Amor were collected from different earthen pots randomly across the three villages from the month of November to December, 2011. The samples were analyzed at Advanced Biological Laboratory of the Biological Sciences Department, University of Agriculture, Makurdi.

SAMPLE ANALYSIS

Serial dilution as adapted by (Chessbroush, 2005). A six fold serial dilution was carried out by measuring 9ml of distilled water into six different test tubes, 1ml of the sample was added to the first test tube initial with 9ml of distill water, making 10ml and 1ml was removed and taken to the next test tube and the same was repeated for the six test tube and on each sample.

MEDIA PREPARATIONS

The media used include: Nutrient agar, potato dextrose agar, MacConkey agar and cystine lactose electrolyte deficient agar. Nutrient Agar: 14g of nutrient agar was dissolved in 500ml of distilled water in a conical flask, stirred thoroughly and allowed to dissolve after which it was sterilized at 121°C for 15 minute using auto clave.

ISOLATION AND IDENTIFICATION OF MICROORGANISMS

The organisms were isolated based on cultural, microbial and biochemical characteristics and also identified based on the shape, size and appearance of colonies formed as described by Chessbrough, 2005.

Biochemical Characteristics

The following biochemical tests were carried out.

Coagulase Test

Colony of test organisms were mixed and properly swirled into drop of plasma on a plain glass slide. Coagulase positive organisms were identified by production of clumps.

Catalase Test

The colony of the test organisms were mixed into a drop of hydrogen peroxide where catalase production was indicated by effervescence.

RESULTS

The results obtained after carrying out the microbiological analysis of water stored in earthen pots from the three host communities of University of Agriculture Makurdi, Benue State were as follow: Table 1 to 3 showed the viable bacterial counts in Shamija, Iorkume and Leva Amor villages respectively and Table 4 to 6 showed the viable fungal counts in Shamija, Iorkume and Leva Amor Villages respectively. Table 7 showed average percentage bacterial count in the three villages collectively while figure 1 is a bar chart showing the frequency distribution of the bacterial count in table 7 above and table 8 shows average percentage fungal viable count in the three villages collectively while figure 2 is a bar chart showing the frequency distribution of fungal count in table 8 above. Table 9 shows the percentage occurrence of bacterial isolate in water stored in earthworm pots in the three villages studied while figure 3 is a bar chart showing the frequency of distribution of bacterial isolated in table 9 above and table 10 showed the percentage occurrence of fungal isolated in water stored in earthen pots in the three villages studied while figure 4 is a bar chart showing the frequency distribution of fungal isolated in table 10 above. The total bacterial count is in the range of 91×10^{-6} to 217×10^{-6} cfu while that of fungal is in the range of 90×10^{-6} to 171×10^{-6} cfu.

Table 1: Shows Specific Bacterial Count in Shamija Village

No of Samples	*Bacillus spp*	*E. coli*	*Klebsiella spp*	*Pseudomonas spp*	*Staphylococcus aureus*	Total
1	2	5	1	2	3	$13x10^{-6}$
2	1	4	0	1	2	$8x10^{-6}$
3	2	3	1	1	3	$10x10^{-6}$
4	0	2	0	1	1	$4x10^{-6}$
5	1	3	0	0	0	$4x10^{-6}$
6	2	2	1	1	0	$6x10^{-6}$
7	0	4	0	0	0	$4x10^{-6}$
8	0	3	0	3	1	$7x10^{-6}$
9	2	8	0	1	3	$14x10^{-6}$
10	0	2	0	0	1	$3x10^{-6}$
11	1	5	0	1	3	$10x10^{-6}$
12	1	6	1	1	4	$13x10^{-6}$
13	2	4	1	0	2	$9x10^{-6}$
14	1	1	0	0	1	$3x10^{-6}$
15	2	2	0	0	0	$4x10^{-6}$
16	0	8	1	1	2	$12x10^{-6}$
17	1	1	0	1	1	$4x10^{-6}$
18	0	4	1	1	2	$8x10^{-6}$
19	3	4	1	1	4	$13x10^{-6}$
20	1	3	0	0	0	$4x10^{-6}$
Total Bacterial	**$22x10^{-6}$**	**$74x10^{-6}$**	**$8x10^{-6}$**	**$16x10^{-6}$**	**$33x10^{-6}$**	**$153x10^{-6}$**

Table 2: Shows total bacterial count in Iorkume village

No. of Sample	*Bacillus* spp	*E.Coli*	*Klebsiella* spp	*Pseudomonas* spp	*Staphylococcus* aureus	Total Cfu/ml
1.	0	2	0	0	1	$3x10^{-6}$
2	2	3	1	1	2	$9x10^{-6}$
3	0	2	0	0	0	$2x10^{-6}$
4	0	3	0	1	2	$6x10^{-6}$
5	0	1	0	0	0	$1x10^{-6}$
6	0	2	0	1	1	$4x10^{-6}$
7	0	3	0	1	0	$4x10^{-6}$
8	0	2	1	1	1	$5x10^{-6}$
9	0	3	0	0	1	$4x10^{-6}$
10	2	2	0	0	1	$5x10^{-6}$
11	0	1	0	0	1	$2x10^{-6}$
12	3	3	0	1	1	$8x10^{-6}$
13	1	2	1	0	0	$4x10^{-6}$
14	0	3	0	0	1	$4x10^{-6}$
15	0	2	0	0	0	$2x10^{-6}$
16	0	2	0	1	2	$5x10^{-6}$
17	1	1	1	0	1	$4x10^{-6}$
18	1	3	1	2	1	$8x10^{-6}$
19	1	1	1	0	1	$4x10^{-6}$
20	1	2	2	1	1	$7x10^{-6}$
Total Bacterial	**$12x10^{-6}$**	**$43x10^{-6}$**	**$8x10^{-6}$**	**$10x10^{-6}$**	**$18x10^{-6}$**	**$91x10^{-6}$**

Table 3: Shows total bacterial count in Leva -Amor village

No of Samples	*Bacillus* spp	*E. coli*	*Klebsiella* spp	*Pseudomonas* spp	*Staphylococcus aureus*	Total
1	3	5	1	3	1	$13x10^{-6}$
2	1	3	1	2	3	$10x10^{-6}$
3	1	4	1	3	1	10×10^{-6}
4	1	4	1	1	4	11×10^{-6}
5	2	8	2	5	1	18×10^{-6}
6	1	4	1	4	2	12×10^{-6}
7	2	5	2	4	4	17×10^{-6}
8	1	3	2	2	2	$10x 10^{-6}$
9	1	3	0	3	2	9×10^{-6}
10	3	4	2	3	2	14×10^{-6}
11	1	3	1	2	3	10×10^{-6}
12	2	2	0	1	1	6×10^{-6}
13	2	5	1	2	4	$14x10^{-6}$
14	1	2	0	1	1	5×10^{-6}
15	1	2	0	0	1	4×10^{-6}
16	0	9	0	3	3	15×10^{-6}
17	0	1	1	0	2	4×10^{-6}
18	1	5	3	2	3	14×10^{-6}
19	1	3	1	2	3	10×10^{-6}
20	1	4	1	1	4	11×10^{-6}
Total Bacterial	**$26x10^{-6}$**	**$79x10^{-6}$**	**$21x10^{-6}$**	**$44x10^{-6}$**	**$47x10^{-6}$**	**$217x10^{-6}$**

Table 4: Shows individual fungal count in Shamija village

No of Samples	*Candida spp*	*Rhizopus spp*	*Mucor spp*	*Penicillin spp*	Total fungal count
1	1	3	1	1	6×10^{-6}
2	2	3	2	1	8×10^{-6}
3	1	1	1	2	5×10^{-6}
4	1	2	1	0	4×10^{-6}
5	1	1	2	2	6×10^{-6}
6	3	4	2	1	10×10^{-6}
7	2	1	1	0	4×10^{-6}
8	1	1	0	0	2×10^{-6}
9	1	5	3	1	10×10^{-6}
10	2	3	1	2	8×10^{-6}
11	1	6	4	1	12×10^{-6}
12	3	5	2	2	12×10^{-6}
13	1	2	0	1	4×10^{-6}
14	1	3	3	1	8×10^{-6}
15	1	3	1	0	5×10^{-6}
16	1	3	2	1	7×10^{-6}
17	1	4	1	4	10×10^{-6}
18	1	3	1	1	6×10^{-6}
19	2	2	1	1	6×10^{-6}
20	0	2	1	0	3×10^{-6}
Total fungal Count	**27×10^{-6}**	**57×10^{-6}**	**30×10^{-6}**	**22×10^{-6}**	**136×10^{-6} cfu/ml**

Table 5: Total and average fungal count in Lorkume Village

No of Samples	*Candida spp*	*Rhizopus spp*	*Mucor spp*	*Penicillin spp*	Total fungal count
1	0	1	1	0	2×10^{-6}
2	0	1	0	1	2×10^{-6}
3	2	4	2	1	9×10^{-6}
4	1	3	1	0	5×10^{-6}
5	1	2	1	1	5×10^{-6}
6	1	1	1	0	3×10^{-6}
7	0	2	0	0	2×10^{-6}
8	2	1	1	0	4×10^{-6}
9	0	2	1	0	4×10^{-6}
10	1	3	1	1	5×10^{-6}
11	1	2	3	2	8×10^{-6}
12	0	3	1	0	5×10^{-6}
13	2	2	0	1	3×10^{-6}
14	1	3	3	1	9×10^{-6}
15	1	1	2	0	4×10^{-6}
16	1	2	1	1	5×10^{-6}
17	1	1	1	0	3×10^{-6}
18	1	4	2	1	8×10^{-6}
19	0	1	1	0	2×10^{-6}
20	0	0	1	1	2×10^{-6}
Total fungal Count	**16×10^{-6}**	**39×10^{-6}**	**24×10^{-6}**	**11×10^{-6}**	**90×10^{-6}cfu/ml**

Table 6: Shows individual fungal count in Leva Amor Village

No of Samples	*Candida spp*	*Rhizopus spp*	*Mucor spp*	*Penicillin spp*	Total fungal count
1	1	4	2	1	8×10^{-6}
2	1	2	3	2	8×10^{-6}
3	0	2	1	1	4×10^{-6}
4	1	5	3	0	9×10^{-6}
5	1	3	4	0	8×10^{-6}
6	0	3	2	1	6×10^{-6}
7	1	5	3	1	10×10^{-6}
8	1	4	4	1	10×10^{-6}
9	2	2	1	1	6×10^{-6}
10	1	1	3	2	7×10^{-6}
11	2	3	3	1	9×10^{-6}
12	2	5	5	2	14×10^{-6}
13	3	2	3	1	9×10^{-6}
14	1	1	1	0	3×10^{-6}
15	0	2	0	2	4×10^{-6}
16	3	3	1	2	9×10^{-6}
17	0	1	2	2	5×10^{-6}
18	1	4	2	1	8×10^{-6}
19	2	8	4	2	16×10^{-6}
20	4	6	6	2	18×10^{-6}
Total fungal Count	**27×10^{-6}**	**66×10^{-6}**	**53×10^{-6}**	**25×10^{-6}**	**171×10^{-6}cfu/ml**

Table 7: Average percentage bacterial count in the three villages studied (Shamija, Iorkume and Leva Amor)

Location	Average number of bacterial count cfu/ml	Total number of samples in each village	Percentage occurrence
Shimaji	8×10^{-6}	20	40%
Iorkume	5×10^{-6}	20	25%
Leva Amor	10×10^{-6}	20	50%

Figure 1: Bar chart showing the frequency of distribution of bacterial count in the village studied

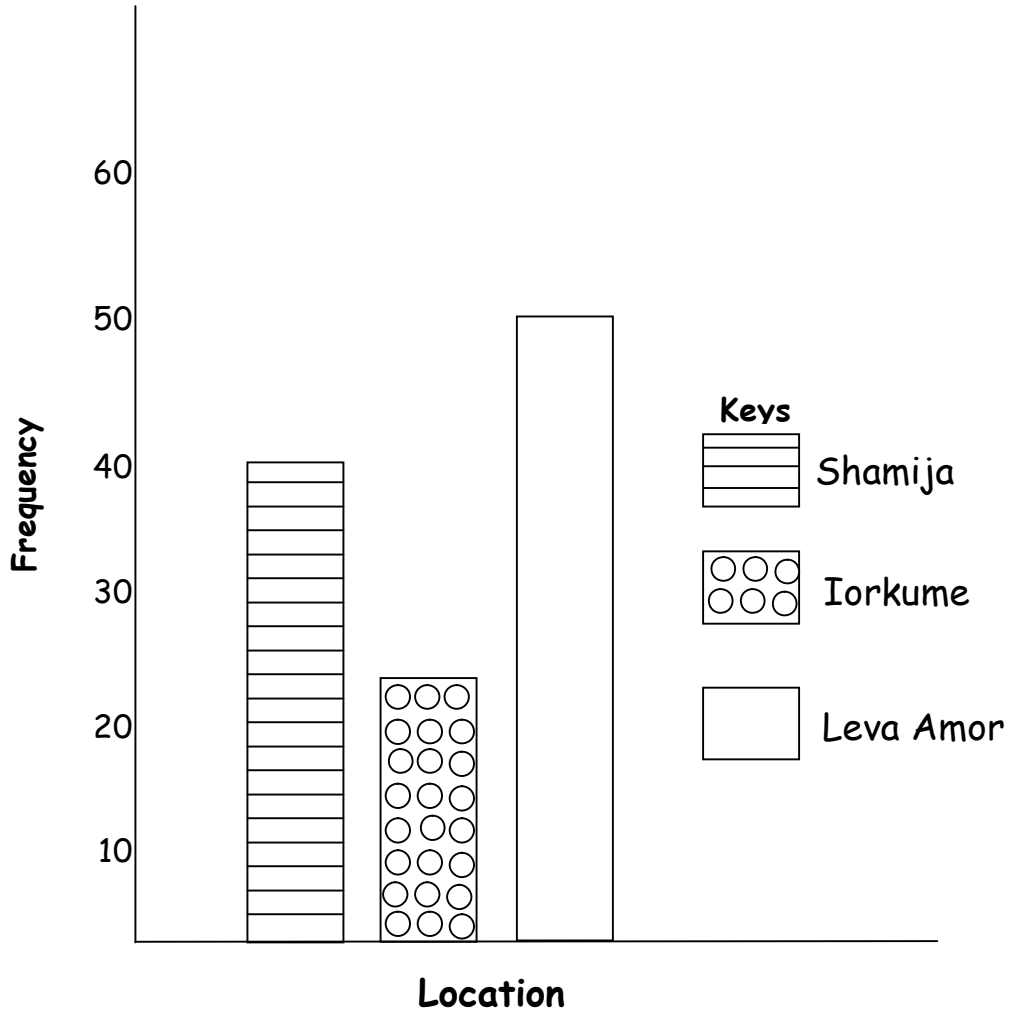


Table 8: Average percentage fungal count in three villages studied (Shamija, Iorkume and Leva Amor)

Location	Average number of fungal count cfu/ml	Total number of samples in each village	Percentage occurrence
Shamija	7×10^{-6}	20	35%
Iorkume	5×10^{-6}	20	25%
Leva Amor	9×10^{-6}	20	45%

Figure 2: Bar chart showing the frequency of distribution of fungal unit in three villages studied

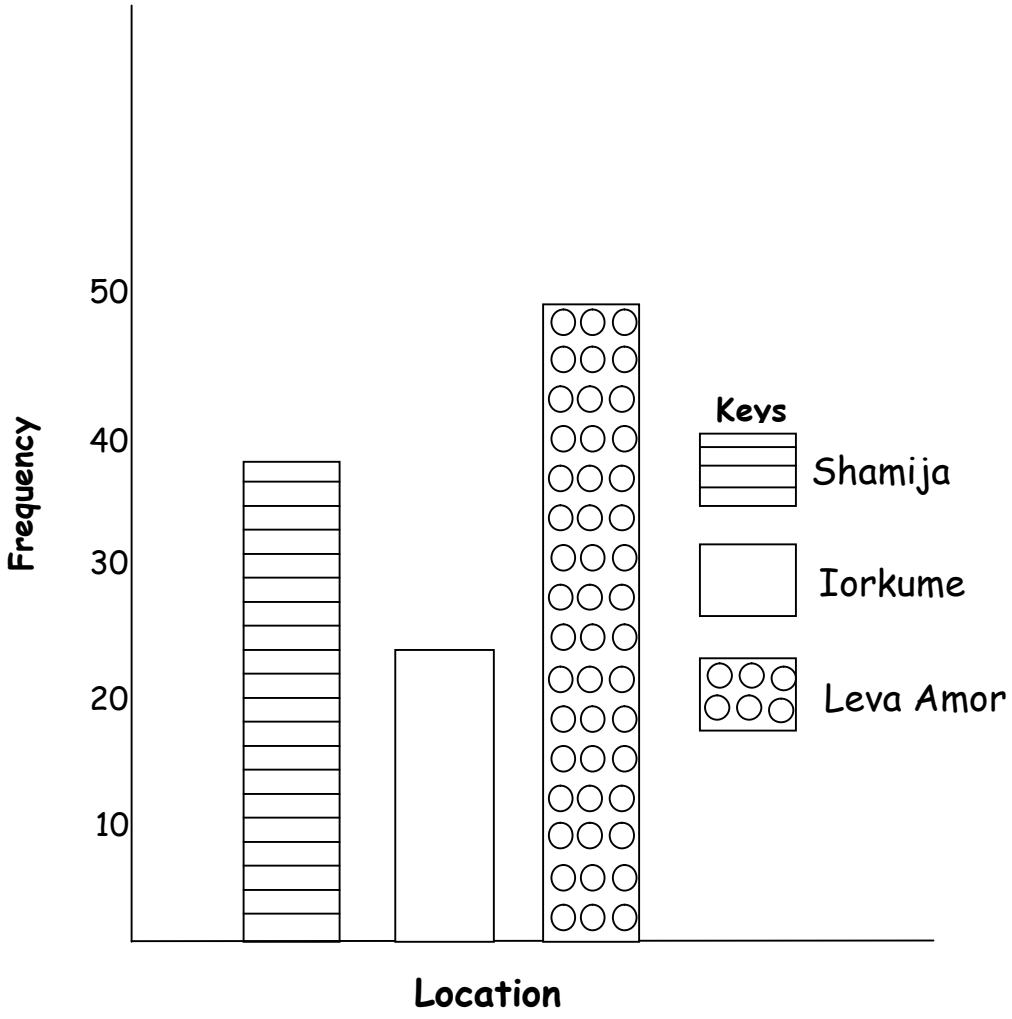
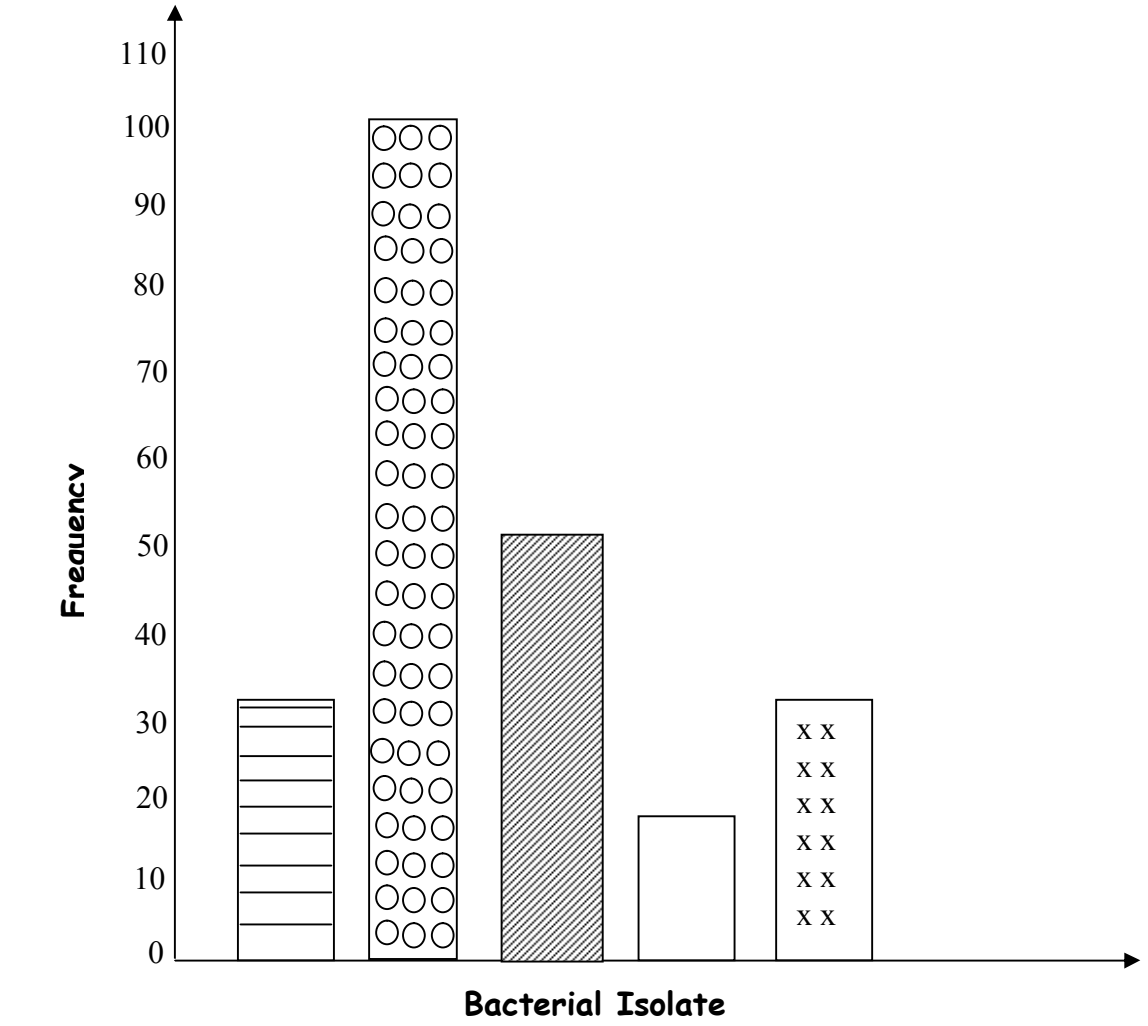


Table 9: Percentage occurrence of bacterial isolate in water stored in earthen pots in three villages studied.

Bacterial Isolates	average number of bacterial isolated	total number sample present	Percentage occurrence
Bacillus spp	20	60	33%
Escherichia coli	65	60	108%
Staphylococcus aureus	33	60	55%
Klebsiella	12	60	20%
Pseudomonas	23	60	38%

Figure 3: Bar chart showing the frequency of distribution of bacterial isolated from water stored in earthen pots in three villages studied.



KEY

Bacillus spp

Escherichia coli

Escherichia coli

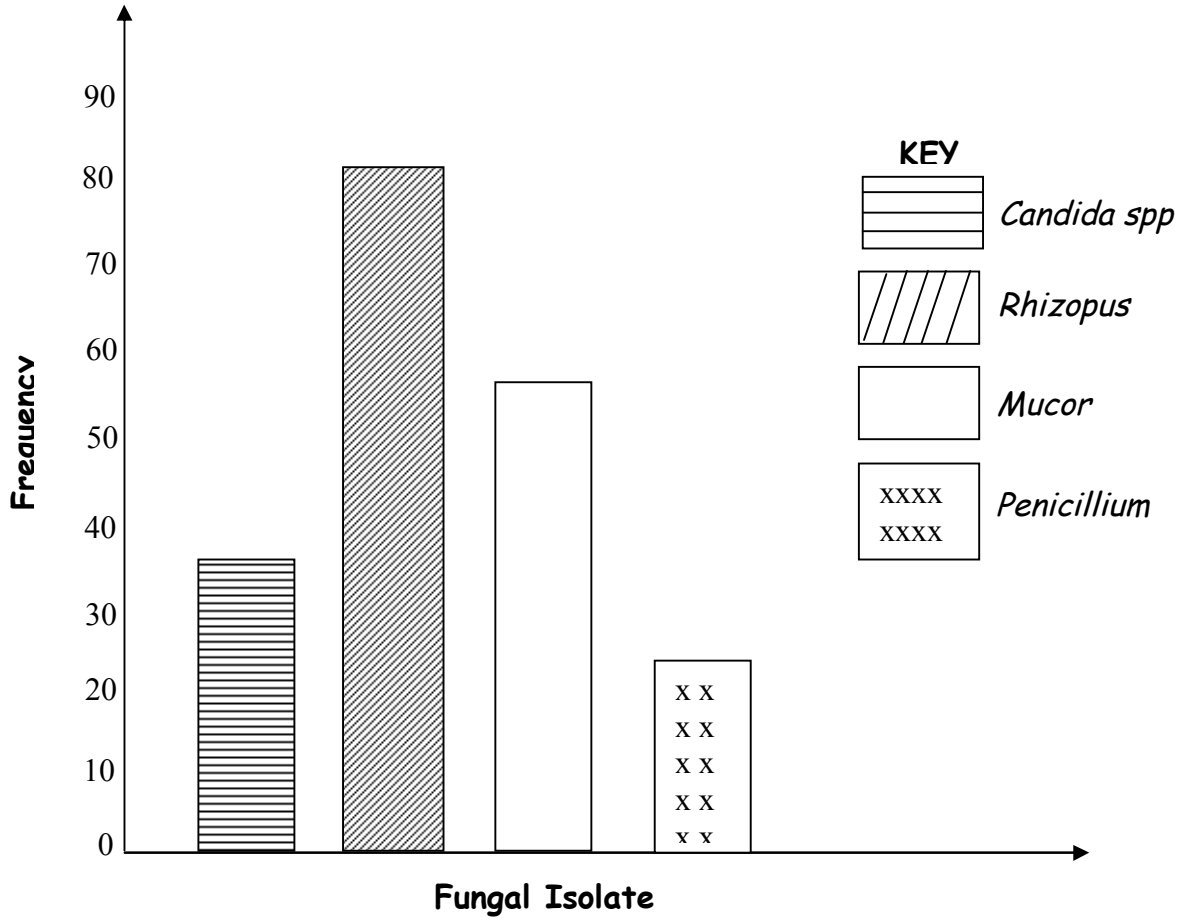
Klebsiella

Pseudomonas

Table 10: Percentage occurrence of fungal isolates in water stored in earthen pots in three villages studied

Fungal isolate	Average number of fungal Isolated	Total number of sample present	Percentage
Candida spp	23	60	38%
Rhizopus	54	60	90%
Mucor	32	60	60%
Penicillium	19	60	32%

Figure 4: Bar chart showing the frequency distribution of fungal isolated from water stored in earthen pots



Discussion

The results obtained from this study showed that the water obtained from earthen pots in these villages was microbial contaminated. Contamination of stored water could be attributed to various factors ranging from the different sources of acquisition, method of collection and household sanitary practices before and after collection. The isolated microorganisms were *Bacillus* spp, *Pseudomonas, klebsiella, Staphylococcus aureaus, Escherichia coli, Candida* spp, *Rhizopus*, *Mucor*, *Penicilliun.* The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environment as was also reported in a study on bacteriological analysis of water obtained from reservoirs in Rawalpiindi Pakistan (2004). According to a study by Togeer Ahmed and Rashida Kanwal (2004) on bacteriological analysis of water obtained from reservoirs, contamination was chiefly from the source of water acquisition as well as poor sanitary conditions of these reservoirs thus exposing this stored water to microorganisms indigenous to the atmosphere. In the three villages studied the result obtained indicated that Shamija and Leva Amor village had a higher level of microbial contaminant, reason could be from source of the water which is either stream or well while the microbial load of Iorkume village is moderate reason been that their source of water is from a treated source which is University of Agriculture Makurdi water works.

Therefore the contamination of water stored in earthen pots in these three village studied was chiefly from the source of water acquisition as well as poor sanitary conditions of these earthen pots as most of the homes in the three villages lacking lids for their earthen pots, thus exposing these stored water to microorganism and sometimes the cup used in scooping the water is contaminated with contaminated hands especially when children frequent the earthen pots. *Klebsiella* and *Pseudomonas* species are opportunistic pathogens, which are naturally present in the environment and are not formally regarded as pathogens: *Klebsiella* was found to originate from potato industry wastewater and other high carbohydrate wastewater. This organism is known to cause pneumonia. Studies in refugee camps in Africa (Paterson *et al.,* 1998) have reported that point of use of disinfection of water in these camps lowered the incidence of pneumonia. *Pseudomonas* is a gram negative, aerobic rod belonging to the family pseudomonadeceae. These bacterial are common inhabitant of soil and water; they breakdown host defence system to initiate infection (Sachs and Mc Arlhur, 2005). *Pseudommas* causes disease such as urinary tract infection, respiratory system infection and gastrointestinal tract infection and a variety of systemic infection. *Bacillus* spp another isolate exist as

an ubiquitous saprophyte in water and air and as such is a major contaminant of water exposed to atmosphere. *Bacillus* can be grown in water and produces enterotoxin which produces poisoning. Poisoning caused by *Bacillus* cereus has two forms, emetic and diarrhea form. The diarrhea type has an incubation period of 1-2hrs and is manifested by profuse diarrhea with abdominal pain, cramp and lever. Each year, more than 2 million persons, mostly children less than five years of age die of diarrhea disease (Kosek *et al.,* 2003; Parashar *et al.,* 2003). For children in this age group, diarrhea disease accounted for 17% of the death from 2000 to 2003 (WHO, 2004) ranking third among causes of death after neonate causes and acute respiratory infections. *Escherichia coli* another bacteria isolated is the most preferred microbial indicator of faecal pollution and is invariably found in humans. Their presence indicates poor sanitary standard (Uraih, 2004). Approximately 40% of all traveler diarrheas are due to infection with enterotoxin form of *Escherichia coli* bacteria. The risk is very high and rises among other things with poor general personal hygiene (CDC, 2004). To prevent contamination hygiene must be monitored carefully in large volume. *Escherichia coli* have also been implicated in urinary tract infection. *Staphylococcus aureus* is another opportunistic pathogen.

It has been reported in more than 50% of healthy humans (Bergdoll, 1990). Then presence in water may cause disease in people with impaired low or general deference mechanisms such as the elderly, young or patients with burns or excessive wounds or those undergoing immunosuppressive therapy or those with AIDS. If people with such ailment use water contaminated with these organisms for drinking or bathing they suffer various infections of the skin and mucous membrane of the eye, ear, nose and throat (WHO, 1993). The pathogenic strain of *Staphyloslococcus aureus* produce enterotoxin A which is specific for mucous membrane of the intensive causing inflammation. It is a protein and is soluble in water and is also heat stable, withstanding exposure to 100°C for a few minutes (Uraih, 2004).

Conclusion

In conclusion, it would be noted that from this study and other similar works cited, contamination of water obtained for storage earthen pots occurred as a result of poor sanitary conditions of the earthen pots, lack of proper lid on the pots, contamination of cups used to scoop water in the pot as well as from source of acquisition.

It is therefore concluded that the use of water obtained from these pots pose a possible health risk to inhabitants of these areas since most of it, if not all homes do not treat these stored water before usage.

Recommendations

Improved sanitation is the first barrier to microbial contamination; therefore individuals should take their personal hygiene seriously. Earthen pots used for storing water should be washed on a regular basis and after collection of water for storage; villages are advice to adult the usage of modified earthen pot approved by a Non-governmental Organization in Kenya called CARE/Kenya, with narrow mouth, lid and a metal tap to prevent contamination by microorganisms indigenous to the atmosphere. According to Hughes and Koplan (2005) among the millennium Development Goals is a call to halve in the year 2015 the proportion of persons without sustainable access to safe drinking water and basic sanitation. Success in reaching this target will help achieve the other goal, increased work force, productivity and substantially reduce the amount of time women and children spend collecting and storing water, which will free them to pursue other productive and educational activities. To this regard the Benue State Government should therefore expedite action on the ongoing Greater Makurdi Water Works Project in order to meet up this challenge both in Makurdi and its environ.

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