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Bacteriological Quality Assessment of Hand-Dug Wells in Mechanic Village Dutse, North-West Nigeria

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ABSTRACT

Background: The need to have constant access to potable water is inevitable to meet one of the goals of the United Nations' sustainable development.
Objectives: This research was conducted to determine the bacteriological quality of the two (2) hand-dug wells available in Dutse mechanic village Jigawa state.
Methods: Twelve (12) water samples were collected from the two (2) hand-dug wells present in the village. The sampling carried out in the morning and afternoon comprised six (6) from well A and six (6) from well B. All the water samples were analyzed for the possible presence and concentration of two waterborne bacteria; Escherichia coli and Klebsiella sp. using the plate count method.
Results: *E. coli* ($10.4x10^6$ CFU/mL) pollution contributed to the highest mean bacterial load detected in well A while *E. coli* ($7.38x10^6$ CFU/mL) pollution was detected in well B. Having subjected the generated results to statistical analysis (T-test at 95% confidence level), there was a significant difference in *E. coli* and *Klebsiella* sp. pollution across the two wells ($p< 0.05$). It is recommended that the wells in the village should be properly given the hygienic practice it deserves by covering them to avert contamination by flies and waterborne pathogens.
Conclusion: *E. coli* and *Klebsiella* sp. were detected in all the water samples assayed in both hand-dug wells. Having a well-kept fetcher devoid of microbial loads and clearing all dump sites close to the wells will ensure microbial-oriented pollution-free well water fit for human consumption.

Keywords: *Escherichia coli*, *Klebsiella sp*., hand-dug well, enteric bacteria, water

INTRODUCTION

It is an undeniable fact that the existence of mankind solely depends on the availability of water for usage as without it, living a comfortable life suffers a great deal. Access to passable safe and potable water is a necessity for communities, unfortunately, countless developing countries in the world over are currently living without stable access to potable water (Obikpo *et al*., 2022). According to several authors (Cosgrove and Rijsberman, 2000; Gomez and Nakat, 2002; UNICEF, 2016), a huge proportion of the developing nations' residents face a whole of problems regarding access to potable water. Strikingly, despite the huge investments that the present and past governments of Nigeria have sunk into an adequate supply of potable water to her citizenry, over 52% of the population have got no access to drinkable water (Oluwasanya, 2009). Regrettably, it has been documented in the literature that most Africans that currently live in villages as well as those who live in cities have no access to clean water meant for drinking (Kassie, 2018; Amoo *et al*., 2021).

Despite the sanitary borehole water provided for the artisans' use in the mechanic village by the Jigawa State government, the regular supply of water got truncated as soon as the power generating set provided got spoilt coupled with the destruction of

electric poles meant for supplying the public electricity to the mechanic village a few years back. The reality on the ground has inevitably sprung the artisans into investing in the construction of hand-dung wells to meet their water needs. However, hand-dug wells have been reported by Ayantobo *et al.* (2012) as being more vulnerable to pollution than those classified as deeper boreholes. United Nations International Children's Emergency Fund (UNICEF) (2021) reported that 86% of Nigerians do not have access to a steady potable water source because municipal water supply is either out of reach or not regular, predominantly in communities that are not that populated.

It has been reported by Amadi *et al.* (2017); Bekuretsion *et al.* (2018); Adeleye *et al*. (2020) that the presence of *Escherichia coli* indicates the presence of waterborne pathogens in any given water. The efficiency of subjecting drinking water to traditional treatment (filtration and chlorination) to remove bacterial pathogens (*Vibrio cholera*, *Salmonella typhi,* and *Salmonella paratyphi*) is well indicated through the adoption of *Escherichia coli* that has been established as faecal indicator bacterium (Edberg *et al.,* 2000; Enriquez *et al.,* 2001). The inevitable need to conduct a bacteriological examination of water to reveal the presence of microorganisms that might cause health hazards has been reported by Singh and Neelam (2011); Adeleye *et al.* (2020).

According to Tya *et al.* (2012), World Health Organization (WHO) has widely recommended that no faecal coliform should be present in 100 mL of water meant for human consumption. These authors reported further that potable water should be odourless, colourless, tasteless, and free from faecal contamination and chemicals above WHO permissible levels. Currently, there are two hundred and twenty-two (222) artisans in the mechanic village who depend on the two hand-dug wells present as their major source of water supply; 2.9% of the artisans admittedly drink water from the wells, 58.6% of artisans utilize the water for ablution, bathing, and other activities while 38.6% artisans utilize the water for cooking and washing of plates (Adeleye *et al.,* 2018). Since the hand-dug wells normally have a low water table, vulnerability to contamination is inevitable (Tya *et al.*, 2012). Owing to these submissions and coupled with the reliance of the majority of the artisans in the village on the two hang-dug wells present for their water needs, this study was carried out to determine the bacteriological quality of the water obtainable therein to ascertain the possible presence of *E. coli* and *Klebsiella* sp.

MATERIALS AND METHODS

Water Assay

Water samples were collected from the two (2) wells present in the study area at two different periods (early morning and mid-afternoon). The first well was labeled as 'A' while the second well was labeled as 'B' respectively. Three (3) water samples were collected from each well making a total of six (6) samples respectively. Clean, dry, and sterile containers were used for collecting each sample to ensure that the samples were devoid of extraneous contaminants as done by Viswanathan (2001); FSSAI (2015).

Containers were sterilized in an autoclave as prescribed by the United State Food and Drug Administration (USFDA) (2018) before sample collection. Having sampled the water in the containers, it was handled with sterile disposable gloves; a fresh glove for each sample. Samples were identified with masking tape labelled legibly with permanent marker. Samples were collected at the beginning of the week as done by the Canadian Food Inspection Agency (CFIA) (2014).

Preparation of Samples

Twenty-five (25) milliliters of each water sample was weighed using a sensitive weighing balance machine as done by Eni *et al.* (2010); Shobha (2014). The samples were serially diluted further into six (6) folds sterile dilution tubes to decrease the population of the organisms sufficiently as done by Begum *et al.* (1986); Waithaka *et al.* (2014). Using the sixth (6th) fold dilution, the first tube for each organism was labeled in respect of the bacterium meant for assessment and the original bacterial culture (OBC), dilution factor. The tubes were subsequently placed on a test tube rack as prescribed by Cappuccino and Sherman (2014).

Using the sixth (6th) fold dilution, the first tube for each organism was labeled in respect of the bacterium meant for assessment and the original bacterial culture (OBC), dilution factor. The tubes were subsequently placed on a test tube rack as prescribed by Cappuccino and Sherman (2014). Using a sterile pipette, one (1) milliliter of the sample was subsequently inoculated into the dilution tube by employing aseptic techniques and mixed thoroughly to obtain 10^1 to 10^6 serial decimal dilution range of the test sample as reported by Prescott (2002); Gopal and Agrawal (2010). The highest decimal dilution (10^6) was then employed for the pour plate process.

Preparation of Culture Media

The culture media used for the isolation and identification of the bacteria were selected based on the particular bacteria being investigated. However, the plate count method (pour plate method) and selective media were employed for the isolation and identification of each of the two organisms being investigated as done by Gopal and Agrawal (2010). The selective medium (MacConkey agar) was prepared by suspending 50 grams of the powder medium in 1000 mL of distilled water, then the medium was heated to dissolve it at 121 °C for 15 minutes. This was subsequently sterilized and allowed cool to 45 °C as reported by Gopal and Agrawal (2010).

Weighing, Dissolution, and Sterilization of Media

A sensitive weighing balance machine was used to measure the amount of the MacConkey agar required for the analysis. The culture media was dissolved and prepared according to the manufacturer's instructions. The MacConkey agar was sterilized immediately after preparation to ensure rapid multiplication of the contaminating bacteria and prevent the composition from being altered as described by Yadav (2012). MacConkey agar was sterilized in an autoclaving machine at 121 °C for 15 minutes.

Inoculation and Incubation

Inoculation was performed in a clean culture room. A sterile graduated pipette was used to transfer 1 mL of the serially diluted sample to a sterile petri dish and approximately, 10 mL of melted agar was added and thoroughly mixed with the sample after it cooled to room temperature as done by Yadav (2012). The agar was allowed to set and the plates were subsequently inverted and incubated at 37 °C for 27 hours for faecal coliform (*Escherichia coli*) and 37 °C for 24 hours for *Kliebsella* as reported by Baveja (2013); Santra (2014); Nishith and Chakraborty (2014).

Identification of Bacteria

Generally, MacConkey agar is the culture medium for the identification of Enterobacteriaceae (Nishith and Chakraborty, 2014). This medium is the selective, differential, and indicator medium used for the culture of *Escherichia coli* due to its ability to distinguish morphologically and biochemically related groups of Enterobacteriaceae. The characteristic change in the appearance of the bacterial growth, medium surrounding the colonies (pink coloration), and the smooth and circular features of *E. coli* growth were used as described by Cappuccino and Sherman (2014); Baveja (2013); Nishith and Chakraborty (2014) in identifying the colonies of the faecal coliform.

As reported by *Rashid and Ebringer (2007), Klebsiella* produces acid, which lowers the pH of the agar below 6.8 and resulted in the appearance of pink colonies. *Klebsiella* produces mucoid colonies which appear very moist and sticky. This phenomenon happens because the bacterium produces capsules.

Colony Enumeration

Having incubated the culture plates at appropriate and acceptable temperature ranges, they were brought out to count the colonies in each petri dish. Counting was done with the help of a colony counting machine as prescribed by Gopal and Agrawal (2010); Eni *et al.* (2010). Since solid media were used for the bacterial culture, colony forming unit (CFU) was adopted in reporting the results as prescribed by Tortora *et al.* (2014). Bacterial counts between 30 to 300 colonies per plate were reported as CFU/mL. Plates with no colonies were reported as <1 CFU/mL and those with colonies >300 were reported as too numerous to count (TNC) as reported by Santra (2014).

Data Analysis

Data collected from this study were analyzed using the computer Statistical

Package for Social Scientists (SPSS) at a 95% probability level of significance. T-test was used to determine the level of significance for the comparison of *Escherichia coli* and *Klebsiella* sp. contaminations between the two hand-dug wells.

RESULTS AND DISCUSSION

The water used in this study comprised six (6) water samples from well A and six (6) water samples from well B. Each sample was analysed for the presence and concentration of *Escherichia coli* (faecal coliform) and *Klebsiella* species. Tables 1(a) and (b) depict the bacterial counts obtained in each sample across the two wells having subjected it to bacterial analysis. The results showed that well A had more bacterial contaminations than well B due to where the well was situated and activities occurring around the well. This can also be a result of fetchers used in drawing water from the well as reported by Nguz *et al.* (2005); Adeleye *et al.* (2018) and might originate from the influence of insect vectors (flies) as the wells were perpetually opened during the conduct of this study.

Klebsiella species were also isolated and counted from the water samples analyzed in which a water sample (A2) from well A (afternoon) recorded the highest *Klebsiella* count ($5.5x10^6$ CFU/mL) and the sample (A2) from well B (morning) recorded the lowest *Klebsiella* count ($2.0x10^5$ CFU/mL). Also, the microbial load in well A was enumerated as seen in Table 2a.

Table 1a: Bacterial counts in well A (reciprocal of dilution = 10^6)

Bacteria	**Water sampling time**					
	Morning (CFU/mL)			Afternoon (CFU/mL)		
	A1	A2	A3	A1	A2	A3
E. coli	$8.0x10^6$	$6.5x10^6$	$7.0x10^6$	$1.4x10^7$	$1.5x10^7$	$1.2x10^7$
Klebsiella sp.	$3.0x10^6$	$1.5x10^6$	$2.5x10^6$	$5.0x10^6$	$5.5x10^6$	$4.0x10^6$

From the results, two water samples (A2 and A1) from well A (afternoon) recorded the highest *Escherichia coli* ($1.5x10^7$ CFU/mL and $1.4x10^7$ CFU/mL) count respectively. While a sample (B2) from well B (morning) recorded the lowest *Escherichia coli* (4.5×10^6 CFU/mL) count as shown in the results.

Table 1b: Bacterial counts in well B (reciprocal of dilution = 10^6)

Bacteria	**Water sampling time**					
	Morning (CFU/mL)			Afternoon (CFU/mL)		
	B1	B2	B3	B1	B2	B3
E. coli	$5.0x10^6$	$4.5x10^6$	$6.0x10^6$	$9.0x10^6$	$9.3x10^6$	$10.5x10^6$
Klebsiella sp.	$1.5x10^6$	$2.0x10^5$	$8.0x10^5$	$3.5x10^6$	$3.0x10^6$	$2.5x10^6$

Table 2a: Loads of enteric bacteria enumerated in well A

Bacteria	**Minimum (CFU/mL)**	**Maximum (CFU/mL)**	**Range (CFU/mL)**	**M (Mean) (CFU/mL)**	**T Value**	**Sig (P-value)**	**Rem**
E. coli	$1.5x10^6$	$6.5x10^7$	$8.5x10^6$	$10.4x10^6$	6.858	0.001	
Klebsiella sp.	$1.5x10^6$	$5.5x10^6$	$4.0x10^6$	$3.58x10^6$	5.736	0.002	

S= Significant

It was discovered that *E coli* had a minimum load of $1.5x10^6$ CFU/mL and a maximum load of $6.5x10^7$ CFU/mL with a range ($8.5x10^6$ CFU/mL and also *E. coli* had a mean load ($10.4x10^6$ CFU/mL). *Klebsiella* sp. was also enumerated from well A, in which the minimum value was enumerated to be $1.5x10^6$ CFU/mL and $5.5x10^6$ CFU/mL as the maximum load with a range ($4.0x10^6$ CFU/mL) together with a mean load of $3.58x10^6$ CFU/mL As reported by Meldrum *et al.* (2009), water meant for human consumption must not present above the recommended microbial count ($>10^2$ CFU/mL). Taking the report of this author into consideration, the results obtained in this current study have clearly shown that all the water samples analyzed in both hand-dug wells for *E. coli* contamination were not satisfactory as they presented way above that should be present in the water meant for human consumption. As reported by Adams and Moss (2008), and Linda (1997), infection may result from ingestion of such contamination levels if the infectious dose falls between 2-1000 cells.

The bacterial load in well B was also enumerated as seen in Table 2b. It can be deduced from the Table that *E coli* had a minimum load ($4.5x10^6$ CFU/mL) and a maximum load ($10.5x10^7$ CFU/mL) with a range of $6.0x10^6$ CFU/mL and also *E. coli* had a mean load of $7.38x10^6$ CFU/mL. *Klebsiella* sp. was also enumerated from well across both periods in which the minimum ($2.0x10^5$ CFU/mL) and maximum ($3.50x10^6$ CFU/mL) loads were enumerated.

From Tables 2a and 2b, it can be deduced that the bacterial load of *E coli* in well A had a minimum, maximum and mean loads of $1.5x10^7$ CFU/mL, $6.5x10^6$ CFU/mL and $10.4x10^6$ CFU/mL respectively can be said to be greater than that of well B. Also, the bacterial load of *Klebsiella* sp. in well A had more minimum ($1.5x10^6$ CFU/mL), maximum ($5.5x10^6$ CFU/mL), range ($4.0x10^6$ CFU/mL) and mean ($1.91x10^6$ CFU/mL) loads than that of well B, thereby making well A more polluted than well B. These results are in tune with the submission of Nguz *et al.* (2005) that attributed the nature of activities around the wells and equipment (fetchers) used in drawing water from the wells as important players that played a significant role in the contamination levels recorded in this current study.

Table 3 shows the bacterial load of the enteric microorganisms across both wells whereby *E coli* had maximum ($4.5x10^6$ CFU/mL), minimum ($1.5x10^6$ CFU/mL), range ($10.5x10^6$ CFU/mL) and mean ($8.9x10^6$ CFU/mL) loads while *Klebsiella* recorded maximum ($5.5x10^6$ CFU/mL), minimum ($2.0x10^5$ CFU/mL), range ($5.3x10^6$ CFU/mL) and mean ($2.75x10^6$ CFU/mL) loads respectively. These results have clearly shown that wells (A and B) have both got loads of enteric *E. coli* and *Klebsiella* sp. The detection of these enteric bacteria in the well water is an indication of recent faecal matter associated contamination (Odonkor and Ampofo, 2013). This phenomenon can trigger undesirable waterborne diseases or public health crisis associated with the detected enteric bacteria as the water obtainable from the wells is consumed. According to the WHO (2017), *E. coli* must record a zero count per 100 mL for such water to be certified harmless for human consumption. Similar detection of enteric *E. coli* and *Klebsiella aerogenes* in drinking water has been reported by Odonkor and Mahami (2020); Falnyi *et al.* (2022). Again, these results have revealed that there was a significant difference in *E. coli* and *Klebsiella* sp. contamination across the two wells. Generally, the results of this study corroborate the findings of Eni *et al.* (2010) which detected higher microbial contamination of 10^5 to 10^8 CFU/mL in their research study. Uzeh *et al.* (2009) also discovered a higher bacteriological load of 10^8 – 10^9 CFU/mL in commonly eaten fresh fruits and vegetables which conforms to this study.

CONCLUSION

The results obtained from the bacteriological analysis indicated that all the water samples examined were highly polluted with *Escherichia coli* and *Klebsiella* sp. No sample from both wells was without any of the two bacterial species isolated and identified in this study. The bacterial loads determined in the water in this study were capable of causing infections attributable to all the bacteria isolated. The fact that *E. coli* pollution was found in the water directly indicates that faecal pollution was evident and, consumers could be at risk of

Table 2b: Loads of enteric bacteria enumerated in well B

Bacteria	Minimum (CFU/mL)	Maximum (CFU/mL)	Range (CFU/mL)	M(Mean) (CFU/mL)	T Value	Sig (P-value)	Remai
E. coli	$4.5x10^6$	$10.5x10^6$	$6.0x10^6$	$7.38x10^6$	7.159	0.001	**S**
Klebsiella sp.	$2.0x10^5$	$3.50x10^6$	$3.30x10^6$	$1.91x10^6$	3.625	0.015	**S**

S= Significant

Table 3: Loads of enteric bacteria enumerated across both wells.

Bacteria	Maximum (CFU/mL)	Minimum (CFU/mL)	Range (CFU/mL)	M (Mean) (CFU/mL)	T Value	Sig p-value	Re
E. coli	$4.5x10^6$	$1.5x10^6$	$10.5x10^6$	$8.9x10^6$	9.013	0.000	
Klebsiella sp.	$5.5x10^6$	$2x10^5$	$5.3x10^6$	$2.75x10^6$	5.926	0.000	

S= Significant

faeco-oral diseases such as Typhoid fever, Gastro-enteritis and Bacillary dysentery which may result in widespread infection or epidemic and pose a public health threat. Also, among the different samples analyzed, *E. coli* had the highest load while *Klebsiella* sp had the lowest load. In addition, the presence of these bacteria in such concentrations indicates that the water in the wells is not properly treated before usage. Improper care given to the wells coupled with the handling of equipment (fetcher and rope) used in drawing water from the wells is other means by which these bacterial species could have caused massive pollution of the wells.

RECOMMENDATIONS

It is recommended that the wells in the village should be properly given the hygienic practice it deserves by covering them at all times to avert pollution by flies and waterborne pathogens. Again, there is an utmost need for the artisans to have a well-kept fetcher devoid of microbial pollutants instead of their current practice of drawing water with multiple fetchers. Additionally, clearing the dump sites close to the wells coupled with the construction of an adequate drainage system and ensuring that the current practice of having dumpsites close to the wells is prohibited will go a long way in ensuring that dumpsite-related microbial pollution is avoided. However, it is important to indicate that this study was limited to the isolation of the enteric bacteria present in the wells investigated. It is recommended that there is a need for further identification of other pathogenic organisms that could be present in the wells and the specific species and strains of the enteric bacteria isolated in this study through the employment of biochemical tests and most importantly molecular identification.

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