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Bacteriological quality of community well water and public health concerns in Enugu urban, Nigeria

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Abstract:

Background: Water is a basic necessity used by humans for both domestic and industrial uses. Next to air, water is essential to life. It takes up about 71% of the earth's surface. The objective of this study is to determine the bacteriological quality of well water in Enugu urban, Nigeria
Methodology: A total of 60 domestic wells were selected from Abakpa, Obiagu and Achara layouts in Engu urban, Nigeria by stratified random sampling method, with 20 wells selected from each area based on location of well sites and construction parameters. Water samples were collected from each well using a sterile 200ml plastic bottle for bacteriological analysis to estimate total bacteria count in colony forming unit (cfu)/ml, total coliform count in most probable number (mpn)/100ml, and faecal coliform count in most probable number (mpn)/100ml. Bacterial isolates were identified using Gram reaction and conventional biochemical tests including catalase and coagulase for Gram positive bacteria, and oxidase, citrate utilization, hydrogen sulfide, indole, urease, methyl red, Voges Proskauer, and sugar fermentation tests for Gram negative bacteria. Antibiotic susceptibility testing (AST) of each isolate was performed by the disk diffusion method against selected antibiotics including penicillin G (10µg), ciprofloxacin (5µg), streptomycin (10µg), amoxicillin-clavulanic acid (20/10µg), and trimethoprim-sulfamethoxazole (25µg), and result interpreted using the European Committee for Antimicrobial Susceptibility Testing (EUCAST) break points. Comparative statistics of the data was performed using analysis of variance (ANOVA) with $p<0.05$ considered statistically significant.
Results: The well water in the three layouts were heavily contaminated as shown by comparatively high mean total bacteria counts of $0.8825\pm0.66x10^4$ cfu/ml, $0.8435\pm0.6413x10^4$ cfu/ml, and $0.8384\pm0.5948x10^4$ cfu/ml for Abakpa, Obiagu and Achara layouts respectively ($p=0.9714$). The mean total coliform counts were 5.15±5.284, 5.45±4.31 and 5.05±4.763 mpn/100ml ($p=0.8038$), and the mean faecal coliform counts were 2.4±3.393, 2.65±2.796 and 2.05±2.35 mpn/100ml ($p=0.9631$) for Abakpa, Obiagu and Achara layouts respectively. A total of 50 pathogenic bacterial isolates were identified; *Klebsiella pneumoniae* 21 (43.8%), *Escherichia coli* 13 (30.0%), *Proteus* spp 6 (12.5%), *Pseudomonas aeruginosa* 6 (12.5%), and *Staphylococcus aureus* 2 (4.2%). The AST result shows that 75% of *K. pneumoniae, E. coli*, *Proteus* spp and *S. aureus* were resistant to all five antibiotics tested.
Conclusion: These findings showed high faecal contamination of domestic well water sources, which poses a significant infection risk to the community. Proper water treatment measures and personal hygiene practices are recommended, and well sites should be located at a safe distance from septic tanks, pit latrines, flowing gutters and refuse dump sites.

Keywords: domestic well water; quality; bacteria pathogens; antibiotic resistance; faecal contamination

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Qualité bactériologique de l'eau de puits communautaire et problèmes de santé publique dans la ville d'Enugu, au Nigeria

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Résumé:

Contexte: L'eau est une nécessité fondamentale utilisée par les humains à la fois pour des usages domestiques et industriels. A côté de l'air, l'eau est essentielle à la vie. Il occupe environ 71% de la surface terrestre. L'objectif de cette étude est de déterminer la qualité bactériologique de l'eau de puits dans la ville d'Enugu, au Nigeria
Méthodologie: Un total de 60 puits domestiques ont été sélectionnés parmi les plans Abakpa, Obiagu et Achara dans la ville d'Enugu, au Nigeria, par une méthode d'échantillonnage aléatoire stratifié, avec 20 puits sélectionnés dans chaque zone en fonction de l'emplacement des sites de puits et des paramètres de construction. Des échantillons d'eau ont été prélevés dans chaque puits à l'aide d'une bouteille en plastique stérile de 200 ml pour une analyse bactériologique afin d'estimer le nombre total de bactéries dans l'unité formant colonie (cfu)/ml, le nombre total de coliformes dans le nombre le plus probable (mpn)/100 ml et le nombre de coliformes fécaux dans la plupart des cas. nombre probable (mpn)/100 ml. Les isolats bactériens ont été identifiés à l'aide de la réaction de Gram et de tests biochimiques conventionnels, notamment la catalase et la coagulase pour les bactéries Gram positives, et l'oxydase, l'utilisation du citrate, le sulfure d'hydrogène, l'indole, l'uréase, le rouge de méthyle, Voges Proskauer et les tests de fermentation des sucres pour les bactéries Gram négatives. Le test de sensibilité aux antibiotiques (AST) de chaque isolat a été effectué par la méthode de diffusion sur disque contre des antibiotiques sélectionnés, notamment la pénicilline G (10 µg), la ciprofloxacine (5 µg), la streptomycine (10 µg), l'amoxicilline-acide clavulanique (20/10 µg) et le triméthoprime-sulfaméthoxazole. (25 µg) et résultat interprété à l'aide des points de rupture du Comité européen pour les tests de sensibilité aux antimicrobiens (EUCAST). Les statistiques comparatives des données ont été réalisées à l'aide d'une analyse de variance (ANOVA) avec $p<0,05$ considéré comme statistiquement significatif.
Résultats: L'eau du puits dans les trois aménagements était fortement contaminée, comme le montrent les taux de bactéries totaux moyens relativement élevés de 0,8825±0,66x10^4 UFC/ml, 0,8435±0,6413x10^4 UFC/ml et 0,8384 ±0,5948x10^4 UFC/ml pour Dispositions Abakpa, Obiagu et Achara respectivement (p=0,9714). Le nombre moyen de coliformes totaux était de 5,15±5,284, 5,45±4,31 et 5,05±4,763 mpn/100 ml (p=0,8038), et le nombre moyen de coliformes fécaux était de 2,4±3,393, 2,65±2,796 et 2,05±2,35 mpn/100 ml (p=0.9631) pour les dispositions Abakpa, Obiagu et Achara respectivement. Un total de 50 isolats bactériens pathogènes ont été identifiés; *Klebsiella pneumoniae* 21 (43,8%), *Escherichia coli* 13 (30,0%), *Proteus* spp 6 (12,5%), *Pseudomonas aeruginosa* 6 (12,5%) et *Staphylococcus aureus* 2 (4,2%). Le résultat de l'AST montre que 75% des *K. pneumoniae, E. coli, Proteus* spp et *S. aureus* étaient résistants aux cinq antibiotiques testés.
Conclusion: Ces résultats ont montré une contamination fécale élevée des sources d'eau de puits domestiques, ce qui pose un risque d'infection important pour la communauté. Des mesures appropriées de traitement de l'eau et des pratiques d'hygiène personnelle sont recommandées, et les sites de puits doivent être situés à une distance de sécurité des fosses septiques, des latrines à fosse, des gouttières et des décharges.

Mots-clés: eau de puits domestique; qualité; bactéries pathogènes; résistance aux antibiotiques; contamination fécale

Introduction:

A well is a type of ground water facility and a major source of water. Modern and treated drilled wells are commonly found in developed countries but in developing countries like Nigeria, with faulty sanitation, access to treated well water is uncommon. This has weakened the health, education and economic activities of the citizens (1,2). In past times, ground water, mostly deep, confined aquifers have been considered to be least susceptible to microbial contamination of human origin. However, protected deep wells can be contaminated where there is a hydrologic connection between these wells and a faulty septic tank or sewer line pit latrines, or contaminated lagoons or rivers. This situation is common in rural and semi-urban environments (3,4).

Access to adequate safe drinking water is an important necessity for every community but many developing regions of the world still lack a steady supply of potable water. Poverty, illiteracy and inadequate sanitary hygiene have also led to an increase in water borne diseases and environmental pollution (5,6). In developing countries, children are the most vulnerable to water borne diseases causing more than 20 million deaths (7). It is speculated that accessible potable water will increase to by only ten per cent in the next thirty years while the earth's population is projected to rise by one third. Except there is an efficient rise in water use, this imbalance will reduce quality water services, diminish the health conditions of the people and deteriorate the environment and globe (8).

Ground water is an age-old alternative source of water used in most households in

Nigeria for domestic purposes. As such, it is a common feature in most residences [9]. A major challenge is the high microbial contaminants found in such wells due to the proximity to contents of septic tanks, open drainages, leachates of dumpsites, flood, soil matter, and agricultural wastes. Inadequate routine disinfection of these wells predisposes an average of 6 to 20 persons (depending on the number of residents) to water borne diseases caused by a variety of pathogenic microbes including enteric bacteria. These microorganisms include *Escherichia coli, Klebsiella* spp*, Shigella, Salmonella, Enterobacter, Citrobacter* spp*, Campylobacter jejuni, Edwardsiella, Hafnia, Serratia, Yersinia, Morganella, Erwinia*, *Providencia* spp, *Pseudomonas* spp*, Proteus* spp*, Acinetobacter* spp*,* and a plethora of other unidentified or unidentifiable bacteria that are transmitted through direct or indirect contamination of water sources by human faeces and waste water (10,11). Some of the diseases caused include dysentery, typhoid fever, paratyphoid fever, infantile paralysis, and hepatitis. These diseases affect about 1.7 billion people worldwide, leading to some 2.2 million deaths annually (12).

The microbiological quality if drinking water is assessed by testing for non-pathogenic bacteria of faecal origin. Microorganisms used as indicators of water quality are coliforms, faecal *Streptococci*, *Clostridium perfringes* and *Pseudomonas aeruginosa* (13). Oguntoke et al., (14) reported that poor well construction, and proximity to point source of contamination make wells vulnerable to microbial pollution. Higher populations of *E. coli* and *Klebsiella* have been found in hand-dug wells during the wet season than during the dry season due to faeces-contaminated flood waters which seep through cracks into the wells (10,15). In line the above reported findings, it was important to determine the microbial quality of domestic wells in three major densely populated areas in Enugu town.

Materials and method:

Study setting and sampling

Randomly selected areas for this study were Abakpa, Obiagu, and Achara layouts, all located in Enugu urban. Enugu is a State in south-east geopolitical zone of Nigeria. It is the 29th largest in area and 22nd most populous, with an estimated population of over 4.4 million as at 2016. The latitude of Enugu, is 6.459964, and the longitude is 7.548949, with GPS coordinates of 6° 27' 35.8704'' N and 7° 32' 56.2164'' E. It is a center of mining, education and commerce. Majority of individuals who reside in these areas are either middle-income or low-income earners both in the public and private sectors of the economy. Well water constitutes the major source of water for domestic purposes in the areas.

Following interactions with the inhabitants, we discovered that wells were constructed with concrete and on the average, are 8 metres deep. Wells for the study were randomly selected, with 20 wells in each layout based on their location (close to septic tank, flooded drainage, dump sites and outdoor sites), and based on wall construction types (good casing, cracked casing, ground level, and ground level with cracked casing).

Collection of water samples

Water samples were collected following the method described by Idowu et al., (16). Water sample from each well was aseptically collected into a sterile 200 ml plastic bottle tied with a strong string to a piece of metal of about 500g. The bottle cap was removed and bottle immersed into the well to a depth of 2 metres. The bottle was then brought up to the surface without touching the sides of the well and was immediately covered. The bottles were placed in cool boxes and transported to the laboratory within 4 hours for analysis.

Total bacteria count

Each of the water samples was serially diluted to a 6-fold dilution. Approximately 9 ml of distilled water was dispensed into 6 labeled tubes. Using a sterile pipette, 1 ml of each water sample was transferred into the first tube. Subsequently, 1 ml was serially diluted into the other tubes. From the 10^{-2} dilutions, 1 ml of water sample was dropped into a sterile Petri dish, and a well-prepared sterile nutrient broth was poured into the Petri dish using pour plate technique. This was properly mixed, allowed to set and incubated at 37°C for 24 hours. The colonies formed were counted and expressed as cfu/ml.

Total coliform and faecal coliform count

The multiple tube fermentation technique described by Collins and Lynne and by American Public Health Association was used (17,18) to determine total and faecal coliform counts. In this method, single and double strength MacConkey broth were prepared in graduated glass flasks, sterilized by autoclaving at 121°C for 15 minutes and dispensed into sterile test tubes containing inverted sterile Durham's tubes. For the presumptive test, from each water sample, 50 ml of water sample was inoculated into a 50 ml double strength MacConkey broth, 10 ml of water sample was inoculated into each of the five 10 ml double strength broth and

1 ml of water sample into each of the five 1 ml single strength broth. The tubes were incubated at 37°C for 24 hours and observed for acid and gas production. Sterile distilled water was used as a control for each test batch.

Presumptive coliform count was determined by the most probable number (MPN) of coliform per 100 ml of water sample using the McCrady's probability tables as reference after combining the various positive and negative results (19). For confirmatory coliform count, a loopful of broth from each positive tube was sub-cultured into a fresh tube of MacConkey broth and incubated at 44°C for 24 hours. Gas production was observed in positive tubes.

Identification of bacterial isolates

Positive tubes from presumptive and confirmatory coliform tests were sub-cultured on Eosin Methylene Blue (EMB) agar for enumeration of faecal coliforms and on nutrient agar. All the inoculated media were incubated at 37°C for 24 hours. Pure isolates were characterized using Gram stain microscopy and conventional biochemical tests as described by Agwaranze et al., (20). The biochemical tests used to characterize Gram-positive isolates were catalase and coagulase tests, while for the Gram-negative isolates, biochemical tests used include oxidase, urease, indole, methyl red, Voges-Proskauer (VP), citrate utilization, h**y**drogen sulfide, and sugar fermentation tests (21).

Antibiotic susceptibility testing

Isolated bacteria were tested against five selected antibiotics; penicillin G (10µg), ciprofloxacin (5µg), streptomycin (10µg), amoxicillin-clavulanic acid (20/10µg), and sulfamethoxazole-trimethoprim (25µg). AST was performed using modified Kirby-Bauer disk diffusion method (22). Briefly, pure colonies of each test bacterium that have been cultured on nutrient agar overnight were used to prepare inoculum in nutrient broth, which was standardized by comparing with 0.5 MacFarland turbidity standards. The inoculum was then streaked on Mueller-Hinton agar plate with a sterile cotton swab. The plate was allowed to dry for 5 min and antibiotics disks were placed on the surface of the agar using sterile forceps. The plates were inverted and incubated aerobically for 24 hours at 37°C. A calibrated ruler was used to measure the diameter of the zone of inhibition around each antibiotic disk. Sensitivity or resistance of each isolate to the antibiotics was determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (23). *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as control strains.

Statistical analysis

Data were presented as mean±SD and analysis was done using GraphPad software (San Diego, USA). Comparison of mean values of variables was done with one way analysis of variance (ANOVA), and *p* value less than 0.05 was considered statistically significant.

Results:

The bacteriological analysis of domestic well water samples shows that in Abakpa, the total bacteria count ranged from 0.05-2.22×10^4 cfu/ml (mean of 0.8825±0.6604 x 10^4 cfu/ml), total coliform count from 0-17 mpn/100ml (mean of 5.15±5.284 mpn/10m) and faecal coliform count of 0-11 mpn/100ml (mean of 2.4± 3.393 mpn/100ml) (Table 1a & 1b). In Obiagu, the mean total bacteria count ranged from 0.06-2.58×10^4 cfu/ml (mean of 0.8435±0.6413x10^4 cfu/ml), total coliform count of 0-13 mpn/100ml (mean of 5.45±4.31 mpn/100ml), and faecal coliform count of 0-9 mpn/100ml (mean of 2.65 ± 2.796 mpn/100ml) (Table 2a & 2b). In Achara layout, the total bacteria count ranged from 0.13-1.86×10^4 cfu/ml (mean of 0.8385±0.5948 x10^4 cfu/ml), total coliform count of 0-17 mpn/100ml (mean of 5.05±4.763 mpn/100ml), and faecal coliform count of 0-7 mpn/100ml (mean of 2.05±2.35 mpn/100ml) (Table 3a & 3b).

Statistical analysis by ANOVA shows that the total bacteria counts were not significantly different between the three layouts (p= 0.9714). Similarly, the total coliform count was not significantly different between the three layouts (p=0.9631) and likewise for the faecal coliform count (p=0.8038).

With respect to the site location of the wells, wells located close to septic tanks (S) had the highest total bacterial and coliform counts followed by wells located close to flooded drainage (F) and dumpsites (DS) while the wells at outdoor (O) sites had the lowest total bacterial counts and did not contain coliforms (Tables 1a, 2a & 3a). With regard to the construction types, wells with cracked casing (CC) had the highest total bacterial and coliform counts followed by wells at ground level (GL) and wells at ground level with cracked casing (GL/CC), while wells with good casing (GC) had the least total bacterial count and had no coliforms (Tables 1b, 2b & 3b).

A total of 50 bacterial isolates were cultured from water samples in the three layouts (Table 4). The isolates were *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus* spp and *Staphylococcus aureus*. *Klebsiella pneumoniae* (43.8%) was the most frequent bacterial isolated while *S. aureus* (4.2%)

was the least frequent bacteria. The bacterial isolates from the wells in the three layouts showed multiple resistance to the antibiotics tested (Tables 5, 6 & 7), except *P. aeruginosa* in Well 4 (S1) in Obiagu (Table 6) and *K. pneumoniae* in Well 2 (F1) in Achara (Table 7), which showed intermediate resistance to sulphamethoxazole-trimethoprim (SXT).

Table 1a: Bacteriological analysis of domestic well water samples in Abakpa from different well sites

Site of well water samples	Total bacteria count (10^4CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Bacteria isolated from samples
S1	2.22	17	0	*Proteus* spp
S2	1.94	13	11	*K. pneumoniae* *E. coli*
S3	1.32	11	8	*E. coli*
S4	0.83	6	4	*E. coli*
S5	0.81	4	4	*E. coli*
F1	1.24	8	0	*Proteus* spp
F2	1.19	9	6	*K. pneumoniae* *E. coli*
F3	2.20	12	7	*K. pneumoniae*
F4	1.06	0	0	*S. aureus*
F5	0.63	9	0	*P. aeruginosa*
DS1	0.95	2	0	*P. aeruginosa*
DS2	0.89	6	4	*K. pneumoniae*
DS3	0.14	0	0	
DS4	0.77	4	4	*K. pneumoniae*
DS5	0.50	2	0	*P. aeruginosa*
O1	0.26	0	0	
O2	0.18	0	0	
O3	0.24	0	0	
O4	0.23	0	0	
O5	0.05	0	0	
Mean count	0.8825	5.15	2.4	
SD	0.6604	5.284	3.393	
Range	0.05-2.22	0-17	0-11	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD = Standard deviation

Table 1b: Bacteriological analysis of domestic well water samples in Abakpa from different well construction types

Samples from different well construction types	Total bacteria count (10^4CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Bacteria isolated from samples
0GC1	0.26	0	0	
GC2	0.18	0	0	
GC3	0.24	0	0	
GC4	0.14	0	0	
GC5	0.05	0	0	
CC1	2.22	17	0	*Proteus* spp
CC2	1.19	9	6	*K. pneumoniae* *E. coli*
CC3	1.32	11	8	*E. coli*
CC4	2.20	12	7	*K. pneumoniae* *E. coli*
CC5	0.23	0	0	
GL1	1.24	8	0	*Proteus* spp.
GL2	1.94	13	11	*K. pneumoniae* *E. coli*
GL3	1.06	0	0	*S. aureus*
GL4	0.50	2	0	*P. aeruginosa*
GL5	0.81	4	4	*E. coli*
GL/CC1	0.95	2	0	*P. aeruginosa*
GL/CC2	0.83	6	4	*E. coli*
GL/CC3	0.89	6	4	*K. pneumoniae*
GL/CC4	0.63	9	0	*P. aeruginosa*
GL/CC5	0.77	4	4	*K. pneumoniae*
Mean count	0.8825	5.15	2.4	
SD	0.6614	5.284	3.393	
Range	0.05-2.22	0-17	0-11	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD=Standard deviation

Table 2a: Bacteriological analysis of domestic well water samples in Obiagu from different well sites

Sites of well water samples	Total bacteria count (10^4CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
S1	1.01	13	6	*E. coli* *P. aeruginosa*
S2	2.58	11	9	*K. pneumoniae*
S3	0.98	9	6	*K. pneumoniae* *E. coli*
S4	1.89	13	8	*K. pneumoniae*
S5	0.69	7	4	*E. coli*
F1	1.15	6	2	*K. pneumoniae* *S. aureus*
F2	0.62	4	2	*K. pneumoniae*
F3	0.70	4	2	*K. pneumoniae*
F4	0.78	4	2	*K. pneumoniae*
F5	0.83	6	2	*K. pneumoniae*
DS1	0.73	4	0	*Proteus* spp.
DS2	0.92	7	4	*E. coli*
DS3	0.86	6	4	*K. pneumoniae*
DS4	1.76	11	0	*Proteus* spp
DS5	0.75	4	2	*K. pneumoniae*
O1	0.17	0	0	
O2	0.12	0	0	
O3	0.11	0	0	
O4	0.06	0	0	
O5	0.16	0	0	
Mean count	0.8435	5.45	2.65	
SD	0.6413	4.310	2.796	
Range	0.06-2.58	0-13	0-9	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD=Standard deviation

Table 2b: Bacteriological analysis of domestic well water samples in Obiagu from different well construction types

Samples from different well construction types	Total bacteria count (10^4CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
GC1	0.17	0	0	
GC2	0.12	0	0	
GC3	0.11	0	0	
GC4	0.06	0	0	
GC5	0.16	0	0	
CC1	1.15	6	2	*K. pneumoniae* *S. aureus*
CC2	1.01	13	6	*E. coli* *P. aeruginosa*
CC3	0.98	9	6	*K. pneumoniae*
CC4	2.58	11	9	*K. pneumoniae* *Proteus* spp
CC5	0.69	7	4	*E. coli*
GL1	0.92	7	4	*E. coli*
GL2	0.86	6	4	*K. pneumoniae*
GL3	0.62	4	2	*K. pneumoniae*
GL4	0.78	4	2	*K. pneumoniae*
GL5	0.75	4	2	*K. pneumoniae*
GL/CC1	0.73	4	0	*Proteus* spp
GL/CC2	1.76	11	0	*Proteus* spp *P. aeruginosa*
GL/CC3	0.70	4	2	*K. pneumoniae*
GL/CC4	1.89	13	8	*K. pneumoniae*
GL/CC5	0.83	6	2	*K. pneumoniae*
Mean count	0.8435	5.45	2.65	
SD	0.6413	4.310	2.796	
Range	0.06-2.58	0-13	0-9	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD = Standard deviation

Table 3a: Bacteriological analysis of domestic well water samples in Achara layout from different well sites

Site of well water samples	Total bacteria count (10^4CFU/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
S1	1.28	11	6	*E. coli*
S2	1.47	17	7	*E. coli*
S3	1.86	6	2	*K. pneumoniae*
S4	0.50	4	2	*E. coli*
S5	0.60	7	4	*E. coli*
F1	1.59	6	4	*K. pneumoniae*
F2	0.76	6	2	*K. pneumoniae*
F3	0.53	4	2	*E. coli*
F4	1.73	12	6	*K. pneumoniae* *E. coli*
F5	0.42	4	0	*Proteus* spp
DS1	1.72	7	0	*P. aeruginosa*
DS2	1.24	2	2	*K. pneumoniae*
DS3	0.79	2	0	*Proteus* spp
DS4	0.89	11	4	*K. pneumoniae*
DS5	0.55	2	0	*P. aeruginosa*
O1	0.18	0	0	
O2	0.21	0	0	
O3	0.15	0	0	
O4	0.17	0	0	
O5	0.13	0	0	
Mean count	0.8385	5.05	2.05	
SD	0.5948	4.763	2.350	
Range	0.13-1.86	0-17	0-7	

S = septic tank; F = flooded drainage; DS = dump site; O = outdoor site; SD = Standard deviation

Table 3b: Bacteriological analysis of domestic well water samples in Achara from different well construction types

Samples from different well construction types	Total bacteria count (CFU/ml×10^4)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Organisms isolated from samples
GC1	0.18	0	0	
GC2	0.21	0	0	
GC3	0.15	0	0	
GC4	0.17	0	0	
GC5	0.13	0	0	
CC1	1.24	2	2	*K. pneumoniae*
CC2	1.28	11	6	*E. coli*
CC3	1.47	17	7	*E. coli*
CC4	0.50	4	2	*E. coli*
CC5	0.60	7	4	*E. coli*
GL1	1.72	7	0	*P. aeruginosa*
GL2	1.59	6	4	*K. pneumoniae*
GL3	0.76	6	2	*K. pneumoniae*
GL4	0.53	4	2	*E. coli*
GL5	0.42	4	0	*Proteus* spp
GL/CC1	0.79	2	0	*Proteus* spp
GL/CC2	0.89	11	4	*K. pneumoniae*
GL/CC3	1.86	6	2	*K. pneumoniae*
GL/CC4	1.73	12	6	*K. pneumoniae* *E. coli*
GL/CC5	0.55	2	0	*P. aeruginosa*
Mean count	0.8385	5.05	2.05	
SD	0.5948	4.763	2.350	
Range	0.13-1.86	0-17	0-7	

GC = good casing; CC = cracked casing, GL = ground level; and GL/CC = ground level with cracked casing; SD=Standard deviation

Table 4: Frequency distribution of bacteria isolates in the domestic well water samples from Abakpa, Obiagu and Achara Layouts, Enugu, Nigeria

Bacteria isolates	**Frequency (%)**
Klebsiella pneumoniae	21 (43.8)
Escherichia coli	15 (30.0)
Pseudomonas aeruginosa	6 (12.5)
Proteus spp	6 (12.5)
Staphylococcus aureus	2 (4.2)
Total	**50 (100)**

Table 5: Antibiotic sensitivity test of isolates from well water samples in Abakpa

Sample	**Isolate**	**Zone Diameter (mm)**				
		CPX	**SXT**	**S**	**PN**	**AU**
S1	*Proteus* spp	8	10	4	6	7
F1	*Proteus* spp	7	9	4	5	9
F2	*K. pneumoniae, E. coli*	8	7	2	3	8
DS1	*P. aeruginosa*	8	5	2	3	2
S2	*K. pneumoniae, E. coli*	9	3	3	5	10
S3	*E. coli*	7	6	4	6	8
F3	*K. pneumoniae*	6	4	2	5	10
S4	*E. coli*	5	6	5	3	7
DS2	*K. pneumoniae*	7	5	3	2	7
F4	*S. aureus*	6	5	2	2	5
F5	*P. aeruginosa*	9	13	5	2	4
DS4	*K. pneumonia*	7	5	3	5	9
DS5	*P. aeruginosa*	6	3	5	2	7
S5	*E. coli*	7	3	4	5	6

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
Antibiotics without breakpoint values have not been determined by EUCAST (2019). The solates were resistant to all the antibiotics by EUCAST guideline

Table 6: Antibiotic sensitivity test of isolates from well water samples in Obiagu

Sample	**Isolate**	**Zone Diameter (mm)**				
		CPX	**SXT**	**S**	**PN**	**AU**
DS1	*Proteus* spp	6	7	4	2	6
DS2	*E. coli*	7	8	3	4	5
F1	*K. pneumoniae, S. aureus*	10	6	6	5	8
S1	*E. coli, P. aeruginosa*	9	13	5	2	4
DS3	*K. pneumoniae*	12	6	7	2	8
DS4	*Proteus* spp	6	6	5	3	8
F2	*K. pneumoniae*	8	4	3	2	9
F3	*K. pneumoniae*	8	6	4	3	6
S3	*K. pneumoniae, E. coli*	7	3	4	4	7
S4	*K. pneumoniae*	14	9	2	5	10
F5	*K. pneumoniae*	7	6	2	1	7
F4	*K. pneumoniae*	7	7	2	3	6
S2	*K. pneumoniae*	13	8	5	2	8
DS5	*K. pneumoniae*	15	7	3	3	7
S5	*E. coli*	7	7	3	3	7

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
Antibiotics without breakpoint values have not been determined by EUCAST (2019). The bacterial isolates were all resistant to the antibiotics except *P. aeruginosa* (Well 4, S1) which showed intermediate resistance to SXT

Table 7: Antibiotic sensitivity test of isolates from well water samples in Achara layout, Enugu, Nigeria

Sample	Isolate	Zone Diameter (mm)				
		CPX	SXT	S	PN	AU
DS1	*P. aeruginosa*	5	5	7	5	6
F1	*K. pneumoniae*	15	11	5	2	8
DS2	*K. pneumoniae*	9	5	7	3	8
F2	*K. pneumoniae*	8	6	5	3	6
DS3	*Proteus* spp	6	5	7	4	6
DS4	*K. pneumonia*	8	8	7	2	7
S1	*E. coli*	5	4	6	7	6
S2	*E. coli*	12	8	4	8	6
S3	*K. pneumoniae*	8	8	5	4	7
S4	*E. coli*	5	7	4	6	7
F3	*E. coli*	9	7	3	6	6
F4	*K. pneumoniae*	10	5	8	4	5
S5	*E. coli*	6	5	6	6	7
DS5	*P. aeruginosa*	7	5	3	5	4
F5	*Proteus* spp	9	8	4	5	8

For *Enterobacteriaceae*: CPX=Ciprofloxacin (5µg): S ≥ 25mm, R < 22mm; SXT=Trimethoprim-sulfamethoxazole (25µg): S ≥ 14mm, R<11mm; S=Streptomycin (10µg): S ≥ -, R < -; PN=Penicillin (10µg): S≥14mm, R< 14mm); AU=Amoxicillin-clavulanic acid (20/10µg): S ≥ 19mm, R<19mm
For *Pseudomonas* spp: CPX: S ≥ 26mm, R<26mm; SXT: S ≥ -, R< -; S: S ≥ 16mm, R< 16mm; PN: S ≥ 18mm, R< 18mm; AU: S ≥ 16mm, R<16mm;
For *Staphylococcus* spp: CPX: S ≥ 21mm, R<21mm; SXT: S ≥ 17mm, R< 14mm; S: S ≥ 18mm, R< 18mm; PN: S ≥ 26mm, R<26mm; AU: S ≥ -, R< -
(EUCAST, 2019). The bacterial isolates were all resistant to the antibiotics except isolates in Well 2 (F1) which showed intermediate resistance to SXT.

Discussion:

The presence of microorganisms determines the sanitary quality of potable water. The guidelines set by the World Health Organization (WHO) approve coliforms, especially *E. coli* as standard indicator organisms. These organisms mainly of faecal origin are pathogenic to man. Ideally, water for drinking, cooking or cleaning purposes should not contain organisms of faecal origin (24). The presence of these bacteria in water is a likely indication of the presence of pathogenic microbes such as bacteria, viruses and parasites. Furthermore, detection of these organisms, especially *E. coli*, in water samples confirms recent faecal contamination, due to the fact that the organism does not survive for long period outside its normal host, which is the gastrointestinal tract of animals (7). The presence of these faecal organisms could be attributed to contamination of the wells by sewage, flooded drainages, poor construction, and poor sanitation.

The findings of high total bacteria count in well water samples from Abakpa (0.05-22.2×10^4 cfu/ml), Obiagu (0.06-2.58×10^4 cfu/ml) and Achara (0.13-1.86×10^4cfu/ml) layouts in our study is similar to those of Onuorah et al., (10) and Agwaranze et al., (20), who reported high coliform counts, indicating that these wells were highly contaminated with bacterial pathogens that can serve as possible cause of water borne diseases. The high bacterial counts may be attributed to run-off water and flooded drainages, which could have possibly entered into some wells during the rainy season and dirt particles from the environment. The total coliform count ranged from 0-17 mpn/100ml in samples from wells in Abakpa, 0-13 mpn /100ml from Obiagu, and 0-17 mpn/100ml from Achara layout. These values exceed the recommended limit of 0 mpn/100ml coliforms in water by the World Health Organization (24).

In the three areas under study, wells located close to septic tanks (S) were grossly contaminated with coliforms while wells located close to dumpsites (DS) and flooded drainages (F) were mildly contaminated, but all the wells at outdoor (O) sites did not contain coliforms. These findings agree with the study conducted in Sagamu by Idowu et al., (16), who reported high numbers of pathogenic organisms of faecal origin from poorly constructed wells. Agwaranze et al., (20) also reported that well water used as source of water for domestic purposes in Wukari was grossly contaminated with faecal coliform bacteria. The siting of the wells close to septic tanks and percolation of sewage inside the wells were possible reasons for contamination by faecal coliforms. Studies in Kenya showed decreased coliform contamination of wells that are situated far from septic tanks (7).

In our study, wells with cracked casing (CC) had the highest bacterial count and coliform contamination, those at ground level (GL) and ground level with cracked casing (GL/CC) also had coliform contamination, but wells with good casing (GC) had no coliform contaminations. These results agreed with the findings of similar studies conducted in Awka (10) and Kaduna (25) in Nigeria where high contaminations of wells by coliforms were reported. The high number of coliforms may be due to the percolation and entry of sewage into ground

water through cracked casings (15), poor construction of the wells and poor environmental conditions of well locations (13). In Ibadan, wells with cracked casings showed high possibilities of contamination from pollutants seeping into them. Furthermore, wells located at ground level were prone to high contamination from materials that gained entrance from the surface.

The frequency distribution of the 50 bacteria isolates is similar to the findings of Agwaranze et al., (20) who isolated *E. coli*, *K. pneumoniae*, *Salmonella typhi, P. aeruginosa, P. vulgaris*, and *S. aureus* from wells in Wukari, Taraba State, Nigeria. Idowu et al., (16) in Sagamu, Ogun State, Nigeria also reported wells that were highly contaminated with faecal coliforms including *E. coli, Klebsiella* spp*, Salmonella* spp, and *Pseudomonas* spp. In microbiological quality assessment of ground water in West Thrace, Turkey, Aydin (26) reported total coliforms, thermo-tolerant coliforms, *E. coli, Salmonella* spp and *P. aeruginosa* isolated in 25%, 17.5%, 15%, 15% and 15% of the ground water samples respectively. Ibiebele and Sokari (27) isolated *E. coli, Enterococcus faecalis, Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus* spp., *Proteus* spp, *Aeromonas* spp, *Chromobacterium* spp, *Flavobacterium* spp, and *Serratia* spp from wells in shanty settlements in Port Harcourt, Nigeria.

The antibiotic susceptibility testing on our isolates showing multiple resistance to antibiotics, is similar to the study of Mishra et al., (28) who reported coliforms from water samples that were resistant to multiple antibiotics. Although bacterial contaminations of the wells in the three layouts were comparable (p=0.9714), wells in Achara and Obiagu had the lower contamination rates than wells in Abakpa. The presence of these pathogenic bacteria in some of the well water samples studied could constitute potential public health hazard to the consumers. High morbidity from enteric diseases such as diarrhoea, dysentery and typhoid fever in Nigeria may be due to widespread consumption of contaminated well water. Therefore, such wells must be adequately treated to protect the health of the consumers.

Conclusion:

Our study showed that domestic wells in Abakpa, Obiagu, and Achara layouts of Enugu urban in Nigeria were highly contaminated by bacteria above the recommended safety levels for domestic water consumption, and presence of coliform bacteria in the water samples indicated recent faecal pollution. Wells located close to septic tanks, dumpsites and flooded drainage, and those with cracked casing and at ground level were more highly contaminated by coliform bacteria. Therefore, to avert the spread of water borne diseases, it is necessary to treat well water before domestic use. Residents of the areas need to be educated on proper hygienic practices and waste disposal. The recommended minimum distance of 15 feet apart between septic tanks, pit latrines, flowing gutters, refuse dump sites and wells should also be observed during well construction.

Conflict of interest:

Authors declare no conflict of interest.

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