Sjournals
Scientific Journals
Scientific Journal of Microbiology (2012) 1(3) 82-90

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Antibiogram of isolated bacteria from Omisanjana hand-dug well water and flowing stream

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ARTICLE INFO

Article history:
Received 30 July 2012
Accepted 25 August 2012
Available online 31 August 2012

Keywords:
Omisanjana stream
Hand dug well
Antibiotic susceptibility
Coliform

ABSTRACT

Water samples were obtained from ten (10) wells around Omisanjana stream, Ado-Ekiti to determine the microbiological and physicochemical quality. The samples were analyzed for the total bacterial and coliform count and the isolated organisms were identified using standard techniques which were further screened for susceptibility to various antibiotics commonly used in the community. The total bacterial count ranged from 2.6 x 10^3 to 10.9 x 10^4 CFU/ml and the total coliform counts ranging from 3.0 x 10^2 to 9.3 x10^4 CFU/ml; which exceeded the WHO standard for drinking water. The organisms isolated belonged to eleven genera, among which *Staphylococcus aureus* showed highest frequency (24.6%) while *Acinetobacter spp*. with least frequency (4.3%). The physicochemical properties of the water accord with the WHO standard with pH range of (6.2-71), temperature (26.9-29.2), turbidity (-0.8 - 6.5) NTU, conductivity (0.04-0.23)µS/cm, total hardness (14-80) mgL^{-1}, TDS (34 to166) mgL^{-1} and TSS of (29 to 122) mgL^{-1}. Higher level of resistance to the antibiotics tested was more prominent in the *E.coli* than in *Staphylococcus aureus*. Although some strains were susceptible to some commonly used antibiotics, but the resistant bacteria encountered pose a serious public health risk especially with the increasing rate of transfer of resistant genes from one bacterium to another. There is therefore need to treat water obtained from wells in the community to make it safe for domestic use.

1. Introduction

Much of the current concern with regards to environmental quality is focused on water because of its importance in maintaining the human health and health of the ecosystem. Fresh water is a finite resource, essential for agriculture, industry and even human existence, without fresh water of adequate quantity and quality, sustainable development will not be possible (Kumar, 1997).

In Nigeria, especially in the rural and sub-urban communities, water for drinking and other domestic uses is mostly obtained from wells dug by inhabitants (Oyetayo *et al*., 2007 and Oluyege *et al*., 2011), in addition to the water available in streams and rivers in rural communities. A well is human-made hole that is dug or drilled deep enough to intersect the water table. If the well is dug beneath the water table, water will fill the open space to the level of the water table, and can be drawn out by a bucket or by pumping (Odeyemi *et al*., 2011). The reliance on untreated ground water (wells) and streams is due to the lack of piped water. Such wells and streams are subject to contamination with pathogenic bacteria because of their proximity to human activities. Also poor wastewater and solid waste management, poor construction and inadequate protection of the wells and presence of latrines close to the wells predispose them to contamination. Fresh water resource becoming day-by-day at the faster rate of deterioration of the water quality is now a global problem (Mahananda *et al*., 2005). Omisanjana stream is an instance of fresh water stream being polluted by dumping refuse constituted mainly of domestic waste. Thereby leading to the deterioration of the water source which might have been seeping and leaching into the nearby shallow wells in the community. Today there is trace contamination not only of surface water but also of groundwater bodies, which are susceptible to leaching from waste dumps, mine tailings and industrial production sites (Moore *et al*., 1998).

The aim of the research work is to study source of contamination of well water and the effect of deteriorated Omisanjana stream have on the neighborhood wells which are closer to the deteriorated flowing stream.

2. Materials and methods

2.1. Collection of water sample

The area of sampling collection is the Omisanjana stream and the hand dug wells which are situated at some distances away from the stream. Omisanjana stream is situated at Omisanjana community in Ado-Ekiti, the capital of Ekiti State, Nigeria. A total of ten water samples were collected from the flowing stream and at different wells around the Omisanjana stream as follow;

Sample A - About 80m to the river at North direction
Sample B - About 120m to the river at North-East direction
Sample C - About 90m to the river at North-West direction
Sample D - About 40m to the river at West-North direction
Sample E - About 70m to the river at South-North direction
Sample F - About 50m to the river at North direction
Sample G - About 70m to the river at South-East direction
Sample H - About 80m to the river at South direction
Sample I - About 40m to the river at East-North direction
Sample J - About 30m to the river at East direction

These samples were collected using 250ml sterile sampling bottles and immediately transported in ice buckets to the Microbiology Laboratory of Ekiti State University, Ado-Ekiti for further work.

2.2. Enumeration of total bacterial counts

Determination of bacterial load in the water samples were done in triplicates. Bacterial plate counts were carried out using the pour plate method with nutrient agar. This method was based on the serial dilution of water sample, which were then pipetted into each sterile Petri-dish. About 20ml of molten nutrient and MacConkey agar

was cooled to 45^{o}C and poured into each Petri-dish containing 1ml of the water sample. Plates were allowed to cool and set after which they are then incubated in inverted position at 37^{o}C. After 24hrs of incubation, the plates were counted by colony counter to obtain the total bacterial counts respectively (Barrow and Feltham, 1993) which was calculated by multiplying the number of colony per plate by the dilution factor and recorded in colony forming unit per ml [CFU/ml] (Olutiola *et al*., 1991).

2.3. Estimation of total coliform count

Using the membrane filtration technique as described by (Olutiola *et al*., 1991), the total coliform count was obtained for water samples by filtering with a membrane filter thereby retaining the bacteria cells on the upper surface of the membrane filter. A 20ml molten MacConkey agar (without salt) plate was poured into an appropriately labeled sterile Petri-dish, cooled to about 45^{o}C and allowed to set. The medium was then inoculated by placing the membrane filter on it, and then inoculated at 37^{o}C for 24hours in an inverted position. After incubation, membrane filter was transferred to an absorbent pad saturated with methylene blue solution (0.01%) and allowed to stain for 1minute. Subsequently, membrane filter was saturated with sterile distilled water so as to remove excess stain. Colonies are counted on membrane filter and used to calculate the number of coliforms per ml of the original sample recorded in CFU/ml unit.

2.4. Antibiotic susceptibility test

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to CLSI (2005). Bacterial isolates were tested against seven ABTEK disc antibiotics which comprised cefotaxime (CAZ 30µg), Cefuroxime (CRX 30µg), Gentamycin (GEN 10µG), Ofloxacin (OFL 5µg), Augmentin (AUG 30µg). Gram negative disc contains additional constituent such as nitrofurantoin (NIT 300µg), ceftazidme (CTX 30µg) and Amoxicillin (AMX 30 µg). Gram positive disc contains additional constituent such as lincomycin (LIN 2µg), oxacilin (OXA 10µg) and cloxacilin (COX 5µg). The inoculum was standardized by adjusting its density to equal the turbidity of a Barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35^{o}C for 18 hours. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline (CLSI, 2005).

2.5. Physicochemical analysis

About 5cm^3 of concentrated HCl was added to 250cm^3 of water sample and evaporated to 25cm^3. The concentrate was transferred to 50cm^3 standard flask and diluted to the mark with distilled de-ionized water (AOAC, 2005). The pH was measured with a KENT EIL 7020 (Kent industrial measurement Limited, Surrey, England) pH-meter, temperature was measured with a simple thermometer calibrated in centigrade, conductivity was measured with CDM83 conductivity meter (By Radiometer A/S Copenhagen, Denmark) after standardization with KCl solution, various standard methods were used for the determination of other parameters. Total alkalinity was determined by acidometry using bromocresol green-methyl red mixed indicator; total hardness and calcium hardness by EDTA titration using Erichrome Black-T-indicator (AOAC, 2005). For the purpose of the samples physical appearance, a visual method of human sense of vision was used. Using the human sense of smelling (nose), the odour of the samples was examined and recorded.

3. Results

The microbiological assessment of ten water samples obtained from different hand-dug well around the Omisanjana stream and the Omisanjana stream in Ado-Ekiti was determined. The total bacteria and coliform counts of the water samples from wells ranged between 2.80 x 10^3 - 65.6 x 10^3 CFU/ml and 0.3 x 10^3 - 51.9 x 10^3 CFU/ml respectively. While water samples from the stream have 30.0 x 10^3 CFU/ml and 24.5 x 10^3 CFU/ml for total bacteria and coliform counts respectively. Total number of one hundred and six (106) bacteria isolates was gotten from well water samples with well H and J having the highest and lowest microbial counts respectively, whereas forty (40) bacteria cells were isolated from water samples from the stream.

Total number of one hundred and forty six (146) of bacteria isolates from the stream and well water samples were characterized and grouped to eleven (11) different genera as follow; *Acinetobacter* spp. and *Flavobacterium* spp have 2.74% each, *Bacillus* spp (4.11%), *Proteus* spp (4.79%), *Klebsiella* spp. and *Shigella* spp. having 6.16%

each, *Salmonella* spp (10.3%), *Enterobacter* spp (11.0%), *Pseudomonas* spp (15.1%), while *Escherichia coli* and *Staphylococcus* spp have 18.5% each (Table 2).

Table 1
Microbial counts (x 10^3 CFU/ml) of water samples.

Water samples	Total Bacteria	Total Coliform
Stream water	30.0	24.5
A	11.9	8.45
B	25.6	15.1
C	8.35	34.9
D	11.8	6.80
E	29.9	21.2
F	18.2	11.5
G	19.6	12.4
H	65.6	51.9
I	27.4	2.20
J	2.80	0.30

A, B, C, D, E, F, G, H, I, and J – Well water samples.

Table 2
Occurrence and percentage distribution of isolates from water samples.

Isolates	Stream water	Occurrence per sample										Total number of isolate	Distribution (%)
		A	B	C	D	E	F	G	H	I	J		
E. coli	12	1	2	2	-	1	1	2	4	2	-	27	18.5
Pseudomonas spp	5	2	1	2	-	2	2	1	3	2	2	22	15.1
Staphylococcus spp.	4	2	2	3	3	2	3	1	3	2	2	27	18.5
Salmonella spp.	5	1	1	1	-	2	1	2	2	-	-	15	10.3
Shigella spp.	3	-	-	-	-	1	-	2	2	1	-	9	6.16
Enterobacter spp.	7	1	1	-	1	-	1	2	2	1	-	16	11.0
Proteus spp.	-	-	1	2	1	1	-	-	1	-	1	7	4.79
Klebsiella spp.	4	1	1	1	-	-	-	-	-	1	1	9	6.16
Bacillus spp.	-	-	-	-	1	3	2	-	-	-	-	6	4.11
Flavobacterium spp.	-	-	-	-	1	1	-	1	1	-	-	4	2.74
Acinetobacter spp.	-	-	1	1	-	-	-	1	1	-	-	4	2.74
Total	40	8	10	13	7	13	10	12	19	9	6	146	100.1

A, B, C, D, E, F, G, H, I, and J – As in Table 1

The percentage antibiotic resistance for isolated Gram negative bacteria were ranged as follow; AUG (60-100%), NIT (57-78%), CAZ (20-100%), CRX (83-100%), GEN (57-100%), CTX (56-100%), OFL (53-100%), AMX (100%) whereas those of Gram positive bacteria ranged as AUG (61-100%), LIN (65-83%), CAZ (0-100%), CRX (60-75%), GEN (0-83%), OXC (50-100%), OFL (0-87%) and OXA (25-100%) (Table 3a and 3b).

The water samples from all sampled wells were observed colourless and odourless except water samples from well H and the stream which were slightly brownish in colour without offensive odour. The pH ranged between 6.2 and 7.3 with well H and D having the least value, while well G has the highest value, whereas the pH value for the stream water sample is 7.25. Temperature of the water samples ranged between 24.5^{o}C and 29.2^{o}C with the stream water sample having the least value. Turbidity of well water samples ranged between 0.08NTU and 6.50NTU with the stream water sample having the value 0.46NTU. Conductivity of the well water samples ranged between 0.04 µScm^{-1} and 0.23µScm^{-1} with the stream water sample having 0.19µScm^{-1}. Total hardness of the well water samples ranged between 14 mgL^{-1} and 80mgL^{-1} with the stream water having 60 mgL^{-1}. Total dissolved solid of the well water samples ranged between 34 mgL^{-1} and166 mgL^{-1} with the stream water having 120

mgL^{-1}. Total suspended solid of the well water samples ranged between 29mgL^{-1} and 122mgL^{-1} with the stream water having 23.27 mgL^{-1}. Acidity of well water samples ranged between 1.73 mgL^{-1} and 1.82 mgL^{-1} with the stream water having 1.80 mgL^{-1}. Alkalinity of well water samples ranged between 22.1 mgL^{-1} and 23.3 mgL^{-1} with the stream water having 22.0 mgL^{-1}. Chloride content of well water samples ranged between 17.1 mgL^{-1} and 22.1 mgL^{-1} with the stream water having 21.3 mgL^{-1}. Sulphate content of well water samples ranged between 5.20 mgL^{-1} and 5.68 mgL^{-1} with the stream water having 5.20 mgL^{-1}. Nitrate value of well water samples ranged between 2.32 mgL^{-1} and 3.50mgL^{-1} with the stream water having 0.50 mgL^{-1}. Phosphate content of well water samples ranged between ND and 0.10 mgL^{-1} with the stream water having 2.50 mgL^{-1} (Table 4).

Table 3a
Antibiotic resistance pattern of isolated Gram negative bacteria from well water samples.

Organisms	Number of isolates	Resistance pattern (%)								Resistotype
		AUG	NIT	CAZ	CRX	GEN	CTX	OFL	AMX	
E. coli	15	87	87	100	100	73	100	53	100	AUG, NIT, CAZ, CRX, CTX, AMX
Enterobacter aerogene	9	100	78	89	89	89	56	78	100	AUG, NIT, CAZ, CRX, GEN, AMX
Klebsiella spp	5	60	60	20	100	60	100	100	100	CRX, CTX, OFL, AMX
Proteus spp	7	100	57	86	100	57	100	71	100	AUG, CAZ, CRX, CTX, AMX
Pseudomonas aeruginosa	17	88	71	100	94	59	100	53	100	CAZ, CRX, CTX, AMX
Salmonella spp	10	70	90	90	100	80	100	70	100	NIT, CAZ, CRX, CTX, AMX
Shigella spp	6	83	67	100	83	100	83	83	100	AUG, CAZ, CRX, GEN, CTX, OFL, AMX

Table 3b
Antibiotic resistance pattern of isolated Gram positive bacteria from well water samples.

Organisms	Number of isolates	Resistance pattern (%)								Resistotype
		AUG	LIN	CAZ	CRX	GEN	OXC	OFL	OXA	
Acinetobacter spp	4	50	75	0	75	50	50	25	25	AUG, CRX, LIN
Bacillus spp	6	83	83	83	67	0	83	0	100	AUG, LIN, CAZ, OXC, OXA
Flavobacterium spp	5	100	80	100	60	60	100	40	100	AUG, CAZ, CTX, AMX
Staphylococcus spp	23	61	65	78	74	83	83	87	96	CAZ, CRX, GEN, OXC, OFL, OXA

CAZ - Ceftazidine, CRX - Cefuroxime, GEN – Gentamycin
OFL - Ofloxacin, AUG – Augmentin, NIT - Nitrofurantoin
CTX - Cefotazine, AMX - Amoxicillin, LIN – Lincomycin
OXC - Oxacilin
R = Resistant, S = Sensitive, I = Intermediate

Table 4

Physicochemical values of water samples from hand dug well and stream in Omisanjana.

Parameter	Stream water	Samples									
		A	B	C	D	E	F	G	H	I	J
Colour	B	CL	CL	CL	CL	CL	CL	CL	B	CL	CL
Odour	NO	OL	OL	OL	OL	OL	OL	OL	NO	OL	OL
pH	6.70	6.5	6.3	6.9	6.2	7.1	7.0	7.3	6.2	6.8	6.4
Acidity (as $CaCO_3$) (mgL^{-1})	1.80	1.80	1.76	1.81	1.73	1.82	1.77	1.77	1.80	1.81	1.82
Total alkalinity (mgL^{-1})	22.0	23.1	23.2	22.1	22.3	20.9	20.7	22.2	23.3	21.5	22.1
Chloride (mgL^{-1})	21.3	22.1	17.5	18.4	19.7	20.3	18.7	17.1	21.3	19.3	21.2
Sulphate (mgL^{-1})	5.20	5.47	5.67	5.65	5.35	5.32	6.32	5.23	5.68	5.35	5.20
Nitrate (mgL^{-1})	0.50	3.50	2.43	3.00	2.61	2.32	3.13	2.40	2.45	3.52	3.45
Phosphate (mgL^{-1})	2.50	0.10	0.10	ND	0.10	ND	ND	ND	0.10	0.01	0.10
Temperature (^0C)	24.5	28.1	28.5	28.0	29.2	27.5	28.0	28.6	29.3	26.9	27.4
Turbidity (NTU)	0.03	0.5	0.08	2.8	1.2	3.0	6.5	0.1	0.8	0.3	2.3
Conductivity ($\mu Scm^{-1} \times 10^2$)	6.3	0.15	0.13	0.04	0.14	0.08	0.05	0.04	0.12	0.23	0.22
Total Hardness (mgL^{-1})	26.5	32	68	40	30	20	14	14	26	80	76
Total dissolved solid (mgL^{-1})	18.26	105	98	34	120	62	38	34.5	85	166	166
Total Suspended Solid (mgL^{-1})	23.27	29	31	37	51	48	54	37	122	48	41
Total solid (mgL^{-1})	41.53	40.5	43.1	39.1	42.2	43.5	41.3	41.7	40.5	41.5	41.7

CL= Colourless, OL = Odourless, NO = Not Offensive, NS = No Standard, B = Brownish

4. Discussion

Omisanjana stream, located in Omisanjana community in Ado-Ekiti is unmanaged surface water that flows around and out of the area to the neighboring community. The pollution rate of the stream due to various human activities cannot be over emphasized. Several wastewater remnants linked up to the stream from nearby household. Refuse were frequently dumped into the stream by the nearby inhabitant of the community. The microbiological and physicochemical analyses conducted on water samples from Omisanjana stream and wells located within the household is a reflection of the rate of contaminations of the wells; thus disclaiming the portability, quality and usability of the water for drinking and other domestic purposes.

The total bacteria count for all the water samples were variably high, exceeding standard limit for microbial count for drinking water (WHO, 2003). The presumed reason for high microbial contamination of the water samples from the river, also account for higher microbial load of the water samples obtained from the well closer to Omisanjana river having higher microbial count compared to that obtained from wells far away from the river. In addition to situation around the stream, the fetcher with which the water is drawn also confers contamination on the well water. Bitton, (1994), reported that surface water runoff and seepage from refuse dumpsite, are the possible source of contamination for ground water. This is because the ground waters are generally believed to be the purest source of water according to Presscott *et al*., (2004). The total coliform count for all the water samples were exceedingly higher as expected, than the maximun contamination level (MCL) for coliform with zero total coliform count per 100ml of water (EPA, 2003). This higher coliform count is an indication that the water sources were faecally contaminated either of human or animal origin (Osuinde and Enezie, 1999). Following the report of Banwo, (2006) who stated that the presence of bushes and shrubs makes it likely possible for animals to be conversant with the source of water for drinking and consequently pass out faeces into the water bodies.

The organisms isolated from the well-water samples around the Omisanjana stream belong to eleven genera, which support the findings of some researchers of related study such as Okonkwo *et al*. (2008) and Odeyemi *et al*. (2011). Isolation of pathogenic organisms such as *Salmonella* spp and *Shigella* spp is of public health significance, having been associated with gastrointestinal infections like dysentery, diarrhoea, typhoid fever and so on (EPA, 2003). The predominate occurrence of *Staphylococcus aureus* in all the water samples is of great importance indicating non-portability of the water based on the production of enterotoxin by this organism (Faria *et al*., 2009). According to Schlegel, (2002), *Enterobacter aerogenes* isolated from the water samples are non-faecal coliform that could be found in vegetation and soil, which further explain how the stream could serve as the source of contamination of the well waters. Following the report of Yagoub and Ahmed, (2010), the isolation of *Pseudomonas aeruginosa*, *Proteus* spp. and other potentially pathogenic bacteria is an indication of water quality deterioration which proves that the immunocompromised people are at great risk; this also signifies a possible connection between the high cases of reported diarrhoea infections in the community.

The results of this study revealed that majority of the gram negative bacteria isolated were resistant to more than three antibiotics. The number of antibiotics to which they were resistant ranged from three (3) to eight (8). Recently, Oyetayo *et al*, (2007) reported the sensitivity of *E. coli* strains from well water in Ondo State, Nigeria to ofloxacin, gentamicin, ciprofloxacin and chloramphenicol with low percentage resistance to these antibiotics similar to the findings of the present study. The sensitivity patterns to the antibiotics used in our investigation were in a very close consonance with the observation of Odeyemi *et al*. (2011), who investigated the bacteriological composition of leachate samples (liquid waste from dumpsite that eventually flows into water bodies) in Ado-Ekiti.

The physicochemical properties recorded for the well water sample could all be considered to be within the range for natural waters according to the standard (WHO, 2003). Variation in pH of the water samples is partially in accordance with Imevbori, (1985) who reported the pH of water bodies to range from 6.5 to 7.4. The low pH value obtained in samples B and H might be due to the high level of free carbon (IV) oxide in the water source relating to the activities around the well as reported by Edema *et al.* (2001). For instance, the low pH value obtained in sample D could be attributed to the production of 'fufu' (a starchy food) very close to the well that serves as the source of the water for the production. This is also in line with the report of John and Mather (1992), who stated that the deviation of pH level of natural water from the neutral is as a result of the CO_2/carbonate/bicarbonate equilibrium. The temperature range obtained is believed to have been influenced by

the intensity of the sunlight as temperature rose from 28^0C to 30^0C on relatively hot days as reported by Banwo, (2006).

The turbidity measured were still in a close range to the EPA standard, which also signified low value obtained for total suspended solid (TSS). Asamo, (2007) related turbidity as the cloudiness of a liquid as a result of particulate matter being suspended within it, and also highlighted that suspended solids helps to shield bacteria. The total dissolved solid enumerated for all the water samples were in line with the EPA standard. High level of TDS observed in sample A, sample D and sample J could be attributed to the chemical used for treatment, since the well for these samples are well casted and often treated as supported by the report of EPA, (2002).

5. Conclusion

This study has shown that different improper activity; illegal refuse dumping, sewage linkage and organic waste disposal carried out on and around the Omisanjana stream has a direct effect on the ground (wells) water used for drinking and other domestic activities in the study area. It is important to emphasize on the fact that many wells water around this community harbor many pathogenic bacteria that have multiple resistant towards some antibiotics. The presence of bacteria most especially, the enteric bacteria which are resistant to antibiotics, poses a serious health hazard to the people of the community. Such organisms can serve as reservoir for antibiotic resistant genes that could be transferred to potentially pathogenic bacteria in the ecosystem. There is therefore an urgent need for awareness to be created about the present situation of their water source and the possible consequence to alert the people of this community of the need to stop all incessant dumping of refuse in and around the river. To orientate the people of the community on the needs for treatment of their well water for consumption and other domestic uses thereby solving the problems that may arise from resistance strains invading the community. Finally, the results from this study also challenge the scientists on the need for more development of new antibiotics to combat the infections caused by these resistance strains.

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